THE AMERICAN JOURNAL OF PHYSIOLOGY

VOLUME LXXII

BALTIMORE, MD.
1925

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

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VOL. LXXII—No. 1

Issued March 1, 1925

BALTIMORE, U. S. A.

1925

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

Made in United States of America

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RELATION BETWEEN BLOOD VOLUME AND BLOOD PRESSURE IN ANAPHYLACTIC AND PEPTONE SHOCK[1]

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Received for publication January 12, 1925

Since the experiments of Heidenhain (1) in 1891, it has been known that the intravenous injection of peptone into a dog caused an increased flow of lymph from the thoracic duct. Starling (2), five years later, showed that the greater part of this fluid comes from the liver and that it is richer in proteid than the lymph that ordinarily escapes from that organ. It is only within recent years, however, that this phenomenon has been considered the cause of the fall in arterial blood pressure which has long been known to follow such injections of peptone into the dog. These two phenomena have been found to occur also in anaphylactic shock in this animal. Accompanying the increased flow of lymph from the liver and the fall in peripheral blood pressure, there is an augmented resistance to the flow of blood through the liver. Manwaring, French and Brill (3) believe that this increased resistance is due to a suddenly increased permeability of the sinusoidal endothelium of the liver which produces an explosive hepatic edema sufficient to cause passive constriction of the sinusoids and hepatic veins. Manwaring, Hosepian and Beattie (4) estimated that the average increase in weight of the dog's liver, exclusive of the contained blood, in peptone shock, represents a withdrawal of 11.7 cc. of plasma from the circulating blood per kilogram of body weight. They believe that this escape of fluid from the circulating blood results in such a reduction in the total blood volume that this is a very important factor in the accompanying fall in peripheral arterial pressure. Peterson and Levinson (5) state that in anaphylactic and peptone shock the sudden stimulation of the endothelium (of the liver) and the associated increase in permeability is quite apparent from a mere analysis of the lymph from the thoracic duct. This is increased in quantity and is richer than normal in protein, and may

[1] Aided by a grant from the Fenger Memorial Fund.

even contain hemoglobin and red blood cells, depending upon the severity of the shock. They also consider the loss of fluid from the circulating blood as it passes through the liver, as an important factor in causing the fall in blood pressure which characterizes these conditions.

There is no question, therefore, that there is a very considerable loss of fluid from the blood in the liver of dogs during anaphylactic and peptone shock. This loss of fluid naturally leads to a concentration of the blood and a reduction of its total volume for a time. Whether a sufficient loss of fluid from the blood can occur within the space of a few seconds to cause the precipitate fall in blood pressure characteristic of this type of shock, is open to question. For peptone and a protein to which a dog has been sensitized, when injected intravenously, produce changes in blood pressure with the speed of adrenalin. It has seemed possible, therefore, that a comparison between the blood volume and blood pressure in anaphylactic and peptone shock might throw some light on this problem. This has been done in a series of somewhat more than twenty dogs, and the results are herewith reported.

The technique used was that described by Lamson (6). Samples of 2 to 3 cc. of blood were taken in a dry syringe from the femoral or jugular veins, before producing shock and at intervals after shock had been induced by the intravenous injection of peptone or a protein to which the dog had previously been sensitized. The drawn blood was placed in dry test tubes containing a small amount of sodium citrate or oxalate to prevent clotting. All of these experiments were carried out under ether anesthesia. The blood pressure was taken by means of a cannula in the carotid artery.

At the conclusion of the experiment upon the animal the several samples of blood were thoroughly shaken. From each tube 0.25 cc. of blood was carefully measured into 25 cc. of distilled water containing 0.04 per cent of ammonium hydroxide. Carbon monoxide gas was then passed through these preparations until all of the hemoglobin had been transformed into carbon monoxide hemoglobin. The sample of blood withdrawn before the induction of shock was taken as the standard in each dog and its volume considered as 100. The samples taken at intervals after the development of shock were compared with this standard in a Duboscq colorimeter, and the volumes of the circulating blood at the time of the taking of each sample were stated as a percentage of the original volume before the injection of the shock-producing substance. The comparisons of the various specimens with the standard were made in duplicate. In a number of cases, the colorimetric readings on the several samples were made also by another person who knew nothing of the history of the specimens he was examining nor of the sequence in which they were taken. The readings by these two persons never yielded a difference greater than 5 per cent in the final calculation.

The results of these experiments fall into four groups. A typical example of each will be given in tabular form.

1. In a majority of non-fatal cases of peptone shock, a definite decrease of blood volume was associated with the fall in arterial blood pressure. The results of a characteristic experiment are shown in table 1.

This experiment, in a dog that recovered from peptone shock, indicates that the blood pressure returns to normal before the blood volume is restored to that present before the injection of the peptone.

2. In most of the fatal cases of peptone and anaphylactic shock there

TABLE 1

Relation of blood volume to blood pressure in group 1

TIME	INJECTION	COLORIMETRIC READINGS	BLOOD VOLUME	BLOOD PRESSURE*
2:20		15.0 (standard)	100	140
2:27		15.0–14.8–15.0	99+	142
2:28	.2 grams peptone			
2:33		12.1–12.0–12.1	81	60
2:43		12.1–12.1–12.1	81	60
2:53		12.6–12.6–12.7	84	75
3:03		13.1–13.0–13.1	87	120
3:23		13.5–13.5–13.5	89	140

* Blood pressure, in millimeters of mercury.

TABLE 2

Relation of blood volume to blood pressure in group 2

TIME	INJECTION	COLORIMETRIC READINGS	BLOOD VOLUME	BLOOD PRESSURE
9:50		10.0 (standard)	100	155
9:52	1.5 grams peptone			
9:53				40
9:57		8.8–8.8–8.9	88	30
10:02		8.1–8.2–8.2	82	15
10:07		7.7–7.7–7.7	77	15

was a reduction of both blood volume and blood pressure without any tendency to improvement in either. Table 2 shows the results of such an experiment.

In these fatal cases the decrease in blood volume and the fall in blood pressure were much greater than in the non-fatal cases. There was no tendency to recovery. The changes in blood volume and blood pressure were not, however, in the same proportion. From table 2 it is seen that in 10 minutes the blood volume had been reduced 18 per cent, while the blood pressure was lowered 93 per cent. In a fatal case of anaphylactic shock the blood volume decreased to 82 while the blood pressure fell to

40 (31 per cent) in five minutes. The animal died in 8 minutes after the injection of the horse serum to which it had been sensitized.

3. In two cases of non-fatal peptone shock there was an actual increase in blood volume. In another dog the blood volume remained practically constant, ranging from 97 to 101. In each of these dogs the usual fall in blood pressure occurred. A typical result is shown in table 3.

TABLE 3

Relation of blood volume to blood pressure in group 3

TIME	INJECTION	COLORIMETRIC READINGS	BLOOD VOLUME	BLOOD PRESSURE
12:05		10.0 (standard)	100	120
12:07	1 gram peptone			
12:08				90
12:12		11.4–11.4	114	110
12:17		11.4–11.3–11.4	114	120
12:18	2 grams peptone			
12:19				60
12:24		11.0–11.0	110	90
12:29				105
12:34		11.9–11.9	119	115
12:39				120
12:44		11.7–11.7	117	120
1:00		11.2–11.2	112	120

TABLE 4

Relation of blood volume to blood pressure when physiological saline is injected to restore blood volume during peptone shock

TIME	INJECTION	COLORIMETRIC READINGS	BLOOD VOLUME	BLOOD PRESSURE
10:55		15.0 (standard)	100	160
10:57	120 cc. NaCl			165
11:05		17.3–17.3	115	165
11:15		16.0–16.0	107	160
11:25		16.2–16.2	108	150
11:35		16.0–16.0	107	145
11:50	1 gram peptone			140
11:52	120 cc. NaCl			50
11:55		16.6–16.6	111	50
12:05		15.5–15.5	103	35

No attempt will be made in this paper to interpret the apparently aberrant results in these three dogs.

4. In the 4th group are those animals in which, during peptone shock, an attempt was made to maintain or to restore blood volume by the injection of salt solution intravenously. In table 4 are shown the results of one such experiment.

The dog used in the experiment shown in table 4 weighed 14 pounds (6.4 kgm.). Lamson and Nagayama (7) have stated that the total volume of blood in the body (of a dog) is in excess of 7.4 per cent of the body weight. Using this figure, the dog used in this experiment had approximately 475 cc. of blood. The injection of 120 cc. of physiological salt solution therefore increased its blood volume about 25 per cent. The first colorimetric determination made on this animal was 10 minutes after the injection of the solution. It showed an increase of 15 per cent in blood volume. Thirty minutes later the blood volume had not yet returned to normal. Almost an hour after the salt solution was given, 1 gram of peptone was injected intravenously, and 2 minutes later another 120 cc. of physiological salt solution was injected into the veins. Table 4 shows that while this dog's blood volume was still 3 per cent above normal its blood pressure was only 35 mm. of mercury.

TABLE 5

Relation of blood volume to blood pressure after injections of hypertonic salt solution in peptone shock

TIME	INJECTION	COLORIMETRIC READINGS	BLOOD VOLUME	BLOOD PRESSURE
11:25		15.0 (standard)	100	154
11:27	2 grams peptone			150
11:30		14.4–14.4	96	50
11:40		13.7–13.7	91	60
11:41	20 cc. 15% NaCl			
11:43		13.5–13.5	90	60
11:46		14.6–14.6	97	50
11:49		15.3–15.3	102	40
11:53		14.3–14.3	95	40

The effect of intravenous injections of hypertonic salt solution was also tried. A characteristic case is shown in table 5.

From these experiments it appears that by the intravenous injection of hypertonic salt solution during peptone shock, the loss of fluid from the circulating blood can be stopped and the blood volume increased, and even actually brought back to normal. This is probably the result of the passage of fluid from the tissues back into the blood vessels. At the same time that this increase in blood volume is occurring, the blood pressure may continue to fall and the animal die from the effects of the extremely low blood pressure.

Discussion. The blood is not a homogeneous fluid, but a suspension of corpuscles whose concentration is known not to be the same throughout the total volume of blood. The term blood volume as used in the experiments described above is admittedly only an approximation. But inasmuch as the conditions were the same throughout each experiment, the

results obtained have a comparative value. The method here used is based upon the amount of hemoglobin in 0.25 cc. of blood—the quantity employed for preparing the samples for colorimetric readings. As Lamson (6) has pointed out "for our present purpose the amount of hemoglobin per unit volume of blood, although inaccurate will suffice to give us an index of variation in the fluid content of the blood."

Manwaring, Hosipean and Beattie (4) attempted to determine the concentration of blood "by following the variations in the circulating red blood cells during canine peptone shock," but considered their results inconclusive. They found that "the circulating red cell count is decreased about 10 per cent during the first minute of peptone shock; . . . . is restored to normal in 6 minutes; and increased above normal by the 12th minute." They believe that one of the factors in the inconclusiveness of red cell counts in the peripheral blood is "a reduction in number of the circulating red blood cells by hepatic sinusoidal stasis." The author found remains of conglutination (stagnation) thrombi in the sinusoids of the livers of dogs 8 and 12 days after repeated injections of peptone (8).

That the method employed in the experiments here reported is reasonably accurate, is indicated by the fact that the results of Manwaring, Hosipean and Beattie (4), obtained by an entirely different method and stated in quite different form, are strikingly like our own when reduced to the same basis. They state that the loss of fluid from the blood represents a withdrawal of 11.7 cc. of plasma per kilogram of body weight. In a dog weighing 10 kilograms, this would mean a loss of 117 cc. of fluid from the circulating blood. If we consider the total blood volume as 7.4 per cent of the body weight (7), and the specific gravity of blood as 1056, such a dog would have approximately 700 cc. of blood. After losing 117 cc. of fluid he would have 83 per cent of the total volume still left in circulation. That this is not very different from our figures, obtained by the colorimetric method, is evident from the tables above.

It is shown by these experiments that the curves of blood volume and blood pressure do not run parallel. This is true not only in those dogs which show a reduction in blood volume during peptone and anaphylactic shock, but also in those few animals in which the volume of blood appears to be increased. By the injection of large quantities of isotonic, and smaller amounts of hypertonic, salt solution, it is possible to maintain or to restore the blood volume to its original level, without inducing any more than a very slight rise in blood pressure while the solution is being injected. It would seem, therefore, that the increase in blood concentration and reduction in total blood volume are not the only factors concerned in the fall in blood pressure in anaphylactic and peptone shock.

SUMMARY

1. In most dogs there is an evident increase in blood concentration and a reduction in blood volume during anaphylactic shock.

2. In a small percentage of dogs there is apparently a decrease in concentration and an increase in blood volume in these conditions in spite of the marked fall in blood pressure.

3. In non-fatal peptone shock the blood pressure regains its normal level before the blood volume is completely restored.

4. Maintaining blood volume at its normal level by the intravenous injection of salt solution does not prevent the fall in blood pressure. Restoring the normal blood volume by the same means does not cause a rise in blood pressure.

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DEVELOPMENTAL STUDY OF EXCITATORY AREAS IN THE CEREBRAL CORTEX OF THE OPOSSUM

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Received for publication January 10, 1925

In an investigation of the developing central nervous system of mammals from a functional and morphological standpoint, the writers (11) first studied the electrical excitability of the cerebral cortex in new-born and very young kittens. It was hoped that the experiments could be extended into the fetal stages of this species but the difficulties of proper control of the physiological condition of the fetus made the results obtained of little value. These technical troubles were largely centered about the maintenance of an adequate blood supply to the brain in fetuses remaining attached to the placenta, or in those entirely separated from the mother. In the latter animals artificial respiration was usually necessary and the general condition of the fetus rapidly became unsatisfactory. Even with decerebration of the mother, with rapid delivery of the fetus still attached to the placenta by the umbilical vessels, and with careful preservation of body heat, experimental stimulation of the fetal cerebral cortex proved unsatisfactory. In large measure, however, these difficulties were found to be technical and it is hoped that refinements in the experimental procedures will yield satisfactory conditions in the fetus for cerebral stimulation.

While in the midst of these technical difficulties it occurred to the writers that by the use of opossums of suitable age many of the experimental conditions would be improved, for in this marsupial the pouch-young afford truly embryonic and fetal forms which are air-breathing and wholly independent of the mother anatomically. It was proposed therefore to subject such pouch-young of various stages to the same experimental procedures which were to be employed in studying the motor cortex in the adult of this species, so that a true basis of comparison between the responses to electrical stimulation in embryo and adult could be had.

As far as the writers are able to determine, credit for the first attempts to determine the motor cortex of the opossum should be given to C. L. Herrick and W. C. Tight (6). Using the bipolar method on one animal, these investigators placed the area for contralateral fore-leg movements

in the region of the orbital sulcus though no very exact data regarding the experimental findings are included in their report. Likewise, these workers reported that an ill-defined area lying 6 to 8 mm. posterior to the orbital sulcus and 4 to 5 mm. from the mid-line, gave on stimulation movements of the hind-leg.

The observations of Ziehen (12) are of particular interest in their relation to the present work, for Ziehen made his experimental determinations on only one opossum—a very young specimen, of which the body length was 23 cm. Using induced currents Ziehen found quite extensive areas for hind-leg, fore-leg and facial movements in the sagittal plane of the cerebral cortex at some distance from the mid-line, the areas occurring in the order named from the posterior to the anterior part of the brain. There was some overlap between the centers for the hind-leg and fore-leg movements, the overlap occurring in the region of the orbital sulcus. The center for the facial musculature required less current than that for the fore-leg which in turn responded to less current than the center for hind-leg movements.

Cunningham (1) was able to locate a definite motor area in the cerebral cortex of three opossums, using constant ether anesthesia and bipolar electrodes with induced current. The chief motor representation occurred along the posterior margin of the orbital sulcus, fore-leg movements being placed near the mid-line and the facial muscles more laterally and just medially to the rhinic fissure. Nasal movements were obtained by stimulation of an area anterior to the orbital sulcus, at the level of the fore-leg movements; similar movements however followed stimulation of the olfactory lobes with the same strong current. In two of the three animals, Cunningham found an area more posteriorly located in the center of the pallium which on excitation gave ear movements. In one animal, movements of the hind-leg were observed on stimulation of the margin of the superior sagittal suture, just behind the fore-leg areas, but as the animal was actually coming out of ether at that time and as the observation could not be repeated, Cunningham assumed that the movement of the hind-leg was really of the spontaneous variety.

In a comprehensive study of the motor cortex in an extensive mammalian series, C. and O. Vogt (10) recorded observations on five South American opossums, D. marsupialis, using ether anesthesia and the unipolar method with induced current. While there was some variation reported in the excitable areas of the different animals, it is apparent that the Vogts obtained isolated movements of the various parts of the facial musculature from the lateral part of the orbital suture, fore-leg movement from the region just posterior to the sulcus near the superior sagittal fissure, and hind-leg (also tail) movements from a rather extensive area midway between the frontal and occipital poles, near the sagittal

suture. The movements of the facial components and of the fore-leg were obtained in all five of the animals; movements of the hind-leg were observed in only four of the five cases. The hind-leg movements recorded by the Vogts were usually described as a movement of the whole leg, though occasionally only the musculature of the foot was involved. And apparently also, the discrimination of finer movements in the fore-leg and in the facial region was quite marked. No differences in the excitability of these various areas to the induced current was noted.

Rogers' (7), (8) observations, recently published in complete form though available for some time in abstracts, differ from the Vogts' in that he was unable to obtain any evidence of areas for hind-leg movements. From small centers, both anterior and posterior to the orbital sulcus, at some distance from the mid-line, movements of the masticatory and facial muscles were obtained; these movements were predominately contralateral but occasionally were ipsilateral. Posterior to the sulcus and near the mid-line, Rogers located areas for the movement of the contralateral fore-leg. In addition Rogers found in the central part of the cortex a large area, stimulation of which caused movements of the vibrissae, the retraction of these hairs being more marked on the opposite side of the body. And finally in the occipital region, Rogers identified a definite area for ocular movements.

An important contribution to this subject has recently been made by Gray and Turner (3), (4), who studied the responses, to unipolar stimulation with induced current, of the cerebral cortex of nine adult Virginian opossums. The experiments were all carefully executed and the histology of the excitable areas well worked out by Gray (2). These authors found a large center for movements of the fore-leg lying just posterior to the orbital suture and just lateral to the superior sagittal fissure; within this large area, smaller centers concerned with more restricted movements of the parts of the fore-leg were made out. Anterior to the orbital sulcus Gray and Turner placed the areas for eye and snout movement but these areas were demonstrable in less than one-fourth of the cases. No center which on stimulation yielded movement of the hind-legs or tail was found, but an extensive area for bilateral movement of vibrissae occurred throughout the region just anterior and lateral to the orbital fissure.

These, then, are the contributions which form the basis on which the present knowledge of the subject of cerebral localization in the opossum rests. Practically all the more recent observers are agreed that the motor areas lie in the region of the orbital fissure but there exists considerable disagreement as to the exact localization of the areas for specific movements. Probably the most interesting question brought forward in this brief résumé of the literature is that of the existence or absence of a

definite center for movements of the hind-leg. The apparent absence of an excitable area for such movements was first noted by Cunningham (1) and his finding has recently been upheld by the observations of Rogers (7), (8) and of Gray and Turner (4). Yet definite responses in the hind-legs were noted by the Vogts in their comprehensive monograph on the subject of cerebral localization; they were recorded also by Ziehen (12) several years before.

With the exception of Ziehen's one animal, which was an immature Virginian opossum, all of the animals used in these studies were adults. The present paper will present additional data in regard to the exact localization of the motor centers in the cerebral cortex of the adult Virginian opossum but will also record the results of similar studies, carried out under identical conditions, on the pouch-young of this species.

METHOD OF EXPERIMENTATION. The animals on which these experiments were performed consisted of eight adults, both male and female, and thirteen pouch-young, ranging in extra-uterine age from 23 days to 89 days. The opossums were all typical examples of Didelphys virginiana and were obtained for the writers through the kindness of Dr. Carl G. Hartman of the University of Texas. The animals were captured in Texas during February, 1924, and were shipped by express to Baltimore. This shipment apparently did not disturb the pregnant females unduly, for good-sized litters were obtained in the laboratory soon after arrival. The female opossums were all kept in individual cages so that all interference with the pouch-young was avoided.

No special preparation of the animals before the experiment was made except that in the case of the adults food was withheld on the day of experimentation. The pouch-young, when used, were first carefully detached from the nipple and were then subjected to the same experimental procedures as were the adults. The animals were all etherized by cone, both adults and pouch-young, and in the haired animals the vertex of the head was shaved. The skin was opened by a mid-line incision extending backward from the glabellar region; the temporal muscles were reflected in the adult and also in the pouch-young when these muscles were developed sufficiently. The skull was entered by using appropriate instruments (trephine and rongeurs when the skull was well ossified; finger forceps in the smaller pouch-young). In the adults, in order to effect a maximal exposure of the cerebral cortex, the zygomatic arch and coronoid process of the mandible were removed, but in the pouch-young practically the whole cerebral cortex could be exposed without removal of these structures. In order to be sure that the underlying brain had not been injured, it was held essential that the removal of the bony calvarium be effected without tearing or damage to the underlying dura. All hemorrhage was controlled so that as far as pos-

sible loss of blood was avoided. The dura was finally opened by cruciate incision and the edges laid back upon the bony skull. The exposed cerebral cortex, still covered by the leptomeninges, was then protected by cotton pledgets saturated with Ringer's solution; before the stimulations were carried out, the cortex was carefully dried by other pledgets in order to avoid as far as possible spread of current.

After these operative procedures were completed, a large indifferent electrode, covered with cotton dampened in strong saline solution, was attached to a shaved area on the side of the animal and the cerebral cortex was explored by a unipolar electrode with spring attachment. Induced current was used throughout, of minimal strength for the elicitation of response. All reactions were carefully noted and plotted with reference to the cerebral landmarks of the individual. At the conclusion of the experiment, the cerebral hemispheres were removed and fixed by immersion in 10 per cent formalin. Subsequently the diagrams used for the initial plotting of the reactions were compared with the fixed specimens.

EXPERIMENTAL FINDINGS. It seems best to present the experimental findings in this report from the developmental standpoint, starting with the youngest of the pouch-young animals and proceeding to the more mature. While this method of presentation possesses certain disadvantages as the motor reactions in this species have been studied by other investigators only in the adult, it permits us to arrive at certain general conclusions concerning the location of the motor areas which have to do with movements of the larger parts of the body and to disregard certain quite obvious individual reactions. One of the most interesting questions regarding the motor cortex of the opossum (the time of initial excitability) we are unfortunately unable to answer, for the earliest of the pouch-young in our series—23 days old—gave very clear-cut and definite responses to the faradic current when applied by the unipolar method.

Pouch-young opossums. This animal, no. 18, 23 days old, with a crown-rump length of 33 mm., was removed from the pouch of the mother by careful traction and was examined for its reactions before etherization. When not disturbed, the animal squirmed around quite vigorously with the more pronounced movements in the fore-legs, though the hind-legs performed well-executed motions. Placed on gauze, the animal grasped the cloth with its fore-legs but did not seem to be able to do this with the hind. All responses to cutaneous stimulation were considered ill-defined because of the constant voluntary movements. The animal was then etherized and the left cerebral cortex was exposed; no orbital fissure could be made out in the pallium. With the usual technique unipolar exploration was then quickly performed. From the points marked *A*, *B*, *C* in figure 1, movements of the contralateral fore-leg were obtained, the predominating factor being an extensor thrust of

the whole leg with outspreading of the toes. Exploration of the rest of the cerebral cortex, with stronger currents, failed to result in any movements of either the facial-masticatory musculature or of the hind-legs. The movements of the fore-legs resulting from stimulation of areas *A*, *B* and *C* along the sagittal fissure were clear-cut and definite.

The next older animal (no. 34) in the series was 41 days old when subjected to experimentation; its crown-rump measurement was 48 mm. After detachment from the nipple of the mother, it was found to have a well-formed mouth and evident vibrissae. The eyes were not yet opened; the hind-legs were not as well developed as were the fore-legs but fairly well-executed movements were made. The animal could not walk with any skill but was able to take a few steps, upholding its body weight.

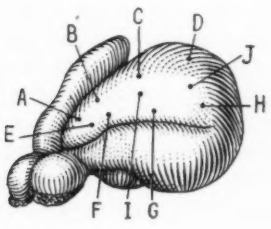

Fig. 1

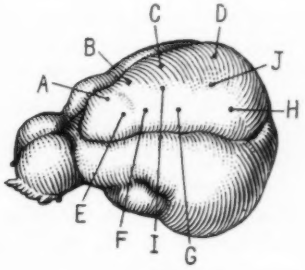

Fig. 2

Fig. 1. Drawing of left cerebral hemisphere of pouch-young opossum, 41 days old, with areas in cortex designated by letters. This drawing will serve as typical of the cerebral cortices of pouch-young opossums before macroscopic differentiation of the orbital sulcus has occurred. × 3.

Fig. 2. Drawing of left cerebral hemisphere of pouch-young opossum, 64 days old, with areas in cortex designated by letters. This drawing may be considered as typical of the cerebral cortices of opossums, soon after macroscopic differentiation of the orbital sulcus has occurred. × 3.

After anesthetization with ether, the whole left cerebral cortex, which showed no obvious orbital suture, was exposed and definite motor responses in the fore-legs were obtained on stimulation of areas *A* and *B*. The responses differed somewhat, excitation of area *A* giving flexion of contralateral fore-leg with spreading of the toes while extension of fore-leg with similar spread of toes followed the excitation of area *B*. All other parts of the cortex yielded no responses even when greatly increased strength of current was employed.

A second pouch-young opossum (no. 38) of the same number of days of age but from another litter was then used. On detachment from the mother the animal was found to have a crown-rump length of 58 mm., and a nose-rump length of 63 mm. The eyes were not opened but the

vibrissae were prominent and a slight downy growth of hair covered its whole body. The fore-legs were more highly developed than the hind and the animal was just able to support its body weight on its legs when taking a few poorly coördinated steps. Areas *A* and *B* (fig. 1) were the only loci in the cerebral cortex which invariably gave motor responses when the brain was explored by the unipolar method. These responses were of the flexor type in the contralateral fore-leg though associated with extension and spreading of the toes; at times slight movements occurred in the ipsilateral fore-leg—apparently due to spread of the current. From area *F*, occasional contractions of the neck musculature were obtained with rotation of the head to the same side. Exploration of the other areas in the exposed cortex failed to show, even with stronger currents, any definite responses.

In this series the next animal (no. 42) was 47 days old when removed from the pouch; it measured 60 mm. from crown to rump and 68 mm. from nose to rump. It was very active, crawling along effectively on all four legs and was able to stand erect though it could not walk with readiness. On removal of the calvarium, after etherization, it was found that for the first time in this series a definitive orbital suture could be identified. From area *A* (fig. 2), movements of the contralateral fore-leg were obtained: flexion at shoulder, elbow, wrist and digits. There was usually abduction of the whole leg during this motor response. On excitation of area *B* (fig. 2), similar movements of the fore-leg were obtained: these were practically identical except that no abduction was observed. The remainder of the cortex revealed on exploration no electrically excitable areas even when stronger currents were employed, no movements of facial musculature or of the hind-leg being observed.

Another animal (no. 45) of the same number of days of age but from another litter, was made use of as a control. This second animal was somewhat larger (crown-rump, 66 mm.; nose-rump, 75 mm.) but in every way the general reactions were identical with the first. On electrical stimulation of the cortex, under ether, similar responses were obtained from areas *A* and *B* (fig. 2), and it was found that the rest of the cerebral cortex was unresponsive even with far stronger current.

Although the next young opossum (no. 51) was 54 days old, its crown-rump measurement was only 62 mm., while its nose-rump was 67 mm. This animal after removal from the pouch was also very active, moving about constantly and supporting its weight largely on its fore-legs which were more developed than the hind. It crawled along fairly well but it seemed unable to make the coördinations necessary for effective walking or running. On exposure of the cerebral cortex under anesthesia, the orbital sulcus was readily identified as a rather shallow furrow. The results of electrical excitation were quite in accord with the findings in the earlier

animals of the series, areas *A*, *B* and *C* (fig. 2), giving definite movement of the contralateral fore-leg. Only the posterior part of area *A*, however, was responsive, and the anterior part of area *C* was likewise the only excitable portion, indicating that the true center for these fore-leg movements was an area somewhat more extensive than that designated area *B*. The manifestations of appropriate stimulation were all flexor in type, the bending occurring at shoulder, elbow and wrist, with slight element of abduction. Quite similar results were obtained also from animal 54, which was 56 days old and measured 74 mm. in crown-rump length and 82 mm. in nose-rump. Area *B* (fig. 2) again was by far the most responsive, the motor reactions from areas *A* and *C* being rather difficult to obtain. In both of these pouch-young opossums, exploration of the

TABLE 1

Responses to faradic stimulation of designated areas in the cerebral cortex of the pouch-young opossum

EXPERIMENT	AGE	CROWN-RUMP LENGTH	AREAS IN CEREBRAL CORTEX					
			A	B	C	D	E	F
	days	*mm.*						
18	23	33	Fore	Fore	Fore			
34	41	48	Fore	Fore				
38	41	58	Fore	Fore				Neck
42	47	60	Fore	Fore				
45	47	66	Fore	Fore				
51	54	62	Fore	Fore	Fore			
54	56	74	Fore	Fore	Fore			
62	62	82	Fore	Fore	Fore?			
70	64	93	Fore	Fore	Fore?			
71	68	96	Fore	Fore				
77	76	127	Face and fore	Fore				
84	82	140	Face and fore	Fore				Face
93	89	157	Face and fore	Fore				Face

rest of the cerebral cortex with strong current failed to elicit contractions of the facial-masticatory musculature or movements of the hind-leg.

It may be noted from table 1 that the next three animals in this series showed practically no differences from those preceding, i.e., the younger animals. Thus, pouch-young no. 62, 62 days old, with a crown-rump measurement of 82 mm. and a nose-rump measurement of 92 mm., gave flexor responses in the contralateral fore-leg on stimulation of area *B* (fig. 2) and to a slighter extent in area *A* while an equivocal movement of the fore-leg was obtained from area *C*. This animal before etherization was found to walk quite readily and to support its weight without difficulty. Opossum 70, a pouch-young animal from litter 3, measured 93 mm. in crown-rump length and 109 mm. in nose-rump length; it was

64 days old when subjected to experimentation. On electrical stimulation of the motor cortex it was found that area *B* (fig. 2) gave the most striking responses, here again an outspoken flexor movement in the contralateral fore-leg; the anterior part of area *C* occasionally gave similar reactions but the posterior portion of area *A* seemed as active in response as area *B*. In pouch-young opossum 71, 68 days old, measuring 96 mm. in crown-rump and 110 mm. in nose-rump length, practically identical reactions were found except that area *C* (fig. 2) was definitely non-responsive. Area *B* was obviously the most active while the posterior part of area *A* again seemed to be, on the basis of motor reactions, an essential part of area *B*. In all of these three animals, from 62 to 68 days of age, no movements of the facial or hind-leg musculature were observed, even on stimulation with strong current.

The next pouch-young animal in the series (no. 77) was 76 days old when used; its crown-rump measurement was 127 mm. and its nose-rump measurement, 151 mm. The animal was out of the pouch and running about the cage, but on being grasped it attempted to crawl back into the pouch. On exposure of the cerebral cortex, under ether, it was found to have a very well-developed orbital suture and to show many of the architectural characters of the adult brain. Electrical stimulation of area *A* (fig. 2) gave, for the first time in this series, definite movements of the facial musculature, the vibrissae, ear and eye being moved on the opposite side. Also from area *A*, entirely from its posterior portion, slight movements of flexion in the contralateral fore-leg were observed. Area *B* showed only outspoken flexor contractions of the musculature of the fore-leg, as observed in the earlier animals in the series. Exploration of the remainder of the cerebral cortex, employing stronger induced currents than were necessary for the responses from areas *A* and *B*, were without motor result.

In the two older animals in the series (no. 84, 82 days old, crown-rump measurement 140 mm., and nose-rump measurement 160 mm.; no. 93, 89 days old, crown-rump measurement 157 mm., and nose-rump measurement 178 mm.) even more marked developmental changes in the motor cortex were observed. From area *A* (fig. 2), the responses were in the facial-masticatory musculature though from the posterior part of this area slight movements of the contralateral fore-leg were obtained. From area *B* all of the reactions following electrical stimulation were flexor movements of the contralateral fore-leg, with bending at shoulder, elbow and wrist, and occasional abduction of the whole extremity. Such findings were quite in accord with the observations on the earlier animals in the series; in one particular, however, the cortex showed a developmental maturity not noted in the preceding specimens. This maturity lay in the fact that stimulation of area *F* gave rise to outspoken

contractions in the contralateral facial musculature, the vibrissae being moved actively and contractions being observed in the exposed temporal muscles. Excitation of all other areas of the cortex, even with strong current, failed to reveal any other motor areas and no movements of the hind-leg or of the tail were noted.

The series of pouch-young opossums, then, whose reactions are given in brief form in table 1, was of interest in demonstrating that even in the youngest form (23 days old) subjected to the experimental procedures, definite responses in the contralateral fore-leg could be obtained. But it was not until the animals reached 76 days of age, that movements in the facial musculature were produced and then from only one area; in the slightly older animals an additional area for facial movements became electrically demonstrable. As these later pouch-young were well able to care for themselves and moved about the cages with rapidity, the reactions of their motor cortices in comparison with those of the adult animals becomes of interest and importance.

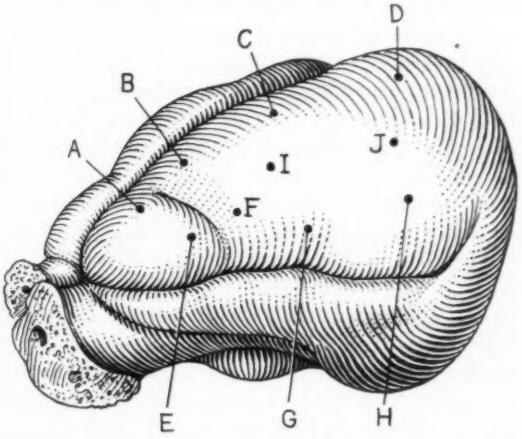

Fig. 3. Drawing of left cerebral hemisphere of adult Virginian opossum, with areas in cortex designated by letters. × 3.

Adult opossums. The number of animals in the series of adult opossums was 8; these were all subjected to experimental procedures as nearly identical as was possible. Considerable variation in the technical difficulties (hemorrhage, etc.) in the adequate exposure of the cerebral cortex occurred, but only results obtained from cortices with wholly unimpaired circulation were considered reliable. In almost all of the opossums, the left cerebral cortex was first exposed and later the right; in every animal the motor responses to electrical excitation of the two sides were identical. The results from the two hemispheres are counted as of one animal and not as of two experiments.

It seems unnecessary to give complete experimental data on the eight animals as the general findings are quite similar in the different individuals and in many respects identical with those in the older pouch-young. Thus from area *A* (fig. 3), movements of the contralateral facial

musculature were obtained with regularity, the contraction of the muscular components being usually associated, though occasionally quite isolated. Movements of the vibrissae were the most readily noted but these were practically always accompanied by contraction of the orbicularis oculi muscle with blinking of the eye. In the movements of the vibrissae the corner of the mouth was retracted, making the phenomenon seem a bilateral one. In those animals in which during the operative procedure the superior auricular muscle was exposed contractions of this muscle were found to follow the movements of the vibrissae, as might be expected from its innervation. But in addition to those rather widespread contractions of the true mimic musculature, there were practically always associated definite contractions of the masticatory group as could be noted from the exposed temporal muscle, and by the frequent quick closure of the mouth and clicking of the teeth. Occasionally during the exploration of this area in an individual animal contractions of only one portion of this facial-masticatory complex of muscles were observed—movement of vibrissae alone, retraction of angle of mouth, movement of ear, etc.—but in all cases these individual movements were represented within the larger area and in general they could not be demonstrated repeatedly but merely at the beginning of the experiment.

From the posterior part of area *A* (fig. 3), movements of the fore-leg were obtained in roughly one-half of the adult opossums. These movements were customarily flexor in type though they were almost always inaugurated by a spreading of the toes. And many times also, particularly after fatigue of the cortex, this spreading of the toes in the contralateral fore-leg was the only motor response to stimulation. And in this posterior portion of area *A* mild contractions of the facial musculature were frequently observed; an overlap of the two areas seemed to be the proper interpretation of the findings.

From area *B* (fig. 3), the motor responses were mainly of the fore-leg—here again, typical flexor movements involving shoulder, elbow and wrist. These movements were very frequently inaugurated by outspreading of the toes, followed quickly by extensive pawing movements of the whole leg. At times the movements were sustained contractions but in the majority of the cases the sustained contraction, even with maintained excitation, was replaced by definite rhythmic motions. In four of the eight animals area *B* was found to represent solely movements of the contralateral fore-leg, but in the other four, excitation of this area, in its anterior portions, resulted in mild contractions of the facial and sometimes of the masticatory musculature, in addition to the definite and outspoken fore-leg movements. This phenomenon was quite similar to the combined fore-leg and facial reactions in the posterior portion of area *A* but in the anterior portions of area *B* the fore-leg element markedly predominated over the facial component.

In addition to these responses from areas *A* and *B* in the adult, area *F* (fig. 3) was found to be definitely motor in every one of the eight animals. The reactions to electrical stimulation of area *F* were almost entirely of the facial-masticatory groups. And occasionally also lateral deviation of the eyes was associated with the contraction of the other muscles of this particular region of the body.

With the exception of these three areas—*A*, *B* and *F* (fig. 3)—exploration of the cerebral cortex of the adult opossum gave negative results. This exploration was always carried out initially with mild induced current of the strength required to evoke typical reactions from the responsive areas; the exploration was then repeated several times with far stronger current and negative results were recorded only when exploration with this stronger current failed to cause motor movements. Particular attention was given to possible movements of the hind-legs and tail: in no case even after stimulation with excessively strong current

TABLE 2

Responses to faradic stimulation of designated areas in the cerebral cortex of the adult opossum

EXPERIMENT	AREAS IN CEREBRAL CORTEX					
	A	B	C	D	E	F
19	Fore	Face				Face
53	Fore	Face and fore				Face
80	Fore	Face and fore				Face
90	Face	Fore				Face
94	Face	Fore				Fore and face
97	Face	Face and fore				Fore and face
98	Face and fore	Fore				Fore and face
103	Face	Fore				Fore and face

were any movements of the hind-legs or of the tail observed. The findings indicated a total absence of centers for these movements. And likewise, in these animals, no evidence of a center for movements of the ocular muscles, definitely located in the occipital region by Rogers (7), (8), was obtained.

The results, then, of the exploration of the cerebral cortex of adult opossums are clear-cut in demonstrating three definite motor areas. The first and third of these (areas *A* and *F*, fig. 3) are primarily concerned with movements of the facial masticatory group of muscles while the second (area *B*) is primarily devoted to the fore-leg. There is, however, in each of these areas some element of representation of the other movement: thus the facial-masticatory areas frequently showed a fore-leg component while the fore-leg area was sometimes involved also in facial-masticatory movements. No areas for hind-leg or for tail were found in any of the animals (cf. table 2).

Discussion. The positive responses of the pouch-young and adult opossum to stimulation of the cerebral cortex with appropriate faradic currents have been recorded in the foregoing sections of this report. It becomes necessary to draw such general conclusions as are possible from these studies and to correlate the findings with those of other workers.

The Virginian opossum, as is well known, gives birth to its young in a very immature condition when these embryonic forms measure about 11 mm. in crown-rump line. As Hartman (5) has recently shown, the embryos when born are not placed by the mother in the pouch but crawl into the pouch, using only their front legs, and quickly become attached to a nipple. At the time of birth, the embryos show fairly well-developed fore-legs but their hind-legs are represented merely by extensive buds. These young then remain in the pouch for 8 to 10 weeks, during the major portion of this time remaining firmly attached to the nipple. Breathing air they represent ideal embryonic material for anatomical and physiological experimentation, inasmuch as they can be removed at will from the pouch of the mother, given anesthesia and subjected to the same experimental procedures as employed for adults.

With such material for experimental use, it becomes obvious that data of value regarding the development of function within the motor portions of the cerebral cortex (as determined by response to electrical stimulation) can be obtained. Even in the earliest pouch-young in this series (23 days old, 33 mm. in crown-rump measurement), indisputable evidence of an area controlling fore-leg movements, contralaterally, was gained. It is unfortunate that younger animals in the series were not used so that the exact time of appearance of these functional centers could be determined but the experimental difficulties in handling, etherizing and operating on such small animals were so great that no attempts on smaller forms were made. In such animals the whole motor area, in the anterior part of the cortex, is so small that the possibility of exact localization becomes extremely difficult. At the time of first demonstration of movement in the opossum following electrical stimulation of the cortex, the animal's eyes are not opened, and great growth of the animal takes place between this stage and the time of opening of the lid slit.

But throughout this period of growth of the pouch-young opossum the motor cortex continues to be excitable, yielding movements of the fore-legs; and it is only when a considerable maturity of the pouch-young is attained that additional centers make their appearance. Thus at an age of 76 days (crown-rump measurement, 127 mm.) the experimental excitations gave evidence of a center for the movements of the contralateral facial-masticatory complex of muscles. And in animals a few days older an additional center, at the lateral part of the orbital suture,

was demonstrated for the same type of facial-masticatory contractions. But at no time throughout the period of pouch life, were centers demonstrable for movements of the hind-legs or the tail.

These experiments, giving evidence of true motor centers existing in the cortex before the opening of the eyes of the animals, add more support to the general conclusion drawn by the writers in another place (11). For in their experiments on new-born and very young kittens, it was shown that a center for fore-leg movements was demonstrable at birth (the kitten's eyes open at 7 to 9 days). These general conclusions are at variance with the original hypothesis of Soltmann (9), who suggested that the assumption of function in motor areas coincided with the initial reception of sensory impulses from eyes and ears. But much of the work carried out by other investigators since Soltmann's publication had led to the belief that the hypothesis of Soltmann, though fascinating, is really without basis of fact.

In other regards also the findings on the pouch-young opossums are of great interest. Careful inspection of the data indicates that initially the area designated *A* functions as a motor area for movements of the fore-leg. At first, with no orbital suture visible macroscopically, the area lies without definite markings in the anterior portion of the cortex but later with the development of a true orbital fissure the area is placed anterior to the sulcus. In the later stages of the pouch-young, only the posterior part of this center lying near to the orbital sulcus seems concerned with movements of the fore-leg, the remainder of the area being devoted to the facial-masticatory musculature. Such an apparent change in function of the area must find its explanation in one or two ways. In the first place it seems most likely that the responsive area as outlined in the youngest animals studied may shift posteriorly so as to be almost entirely posterior to the sulcus; this view does not appear entirely supported by the results of stimulation on the pouch-young of intermediate age where the initial markings of the orbital sulcus are already indicated. The continued growth of the hemispheres, however, results in the further pushing of the area posteriorly. On such a basis, the growth of the hemispheres, with consequently shifting of the essential cellular elements would probably be the best explanation of the phenomenon. A possible second explanation may be based on a change in the function of the area so that the cells which originally had most to do with the fore-leg, in later development, came to assume a similar function in regard to the facial-masticatory musculature. It is not felt that there is any evidence, at hand or in the modern conceptions of neurological growth or function which supports in any way this hypothesis. Far more likely is it that, if the initial explanation of growth-shifting be not correct, the responses from excitation of the anterior area (*A*) are due to spread of current from

the primary center for fore-leg movement which is situated more posteriorly throughout life. The overlap, however, of the areas for face and fore-leg indicates in all of these developmental studies a very close association of the two functions.

When attention is given to the distribution of the motor areas in the brain of the adult opossum, as determined in these studies, it is found that considerable variation exists between the present findings and those of previous investigators. It is quite impossible to correlate our observations with those of Herrick (6) or those of Ziehen (12) but with the motor areas given by Cunningham (1) our findings are quite in agreement except that in this series of adult opossums it has never been found possible to demonstrate the posteriorly located center for ear movements. In general the centers for fore-leg and for facial movements correspond closely, as determined on these eight opossums, to the general areas for these movements worked out by the Vogts (10) though the observations of these authors are replete with detailed specific individual movements. Yet by homologizing the centers of specific movements with the gross part of the body in which they are located, Vogt's diagrams would answer fairly well for our series, except that at no time during our experiments were we able to demonstrate any area which on stimulation gave rise to movements of the hind-leg or tail.

It is possible to correlate in a broad way our findings with those recently reported by Rogers (7), (8) though in some respects divergencies occur. The areas for fore-leg movement as delimited by Rogers lie near the mid-line just posterior to the orbital sulcus; these areas correspond to those designated *B* in our series. Anterior to the sulcus, Rogers found areas for facial-masticatory movement, extending laterally and slightly posterior. In part these correspond to our areas *A* and *F*. But we were not able to verify Roger's observation that in the central part of the hemisphere there existed a large area for movements of the contralateral and ipsilateral vibrissae and also for the ipsilateral temporal muscle. We would relate such movements of vibrissae to contractions of the mimic musculature; the possibility of the ipsilateral temporal movement being due to spread of current seems really very great.

The observations of Gray and Turner (3), (4) vary in some particulars from our own findings but the variations are not apparently of great significance. Movements of the fore-leg were obtained by these workers on stimulation of the area just posterior to the orbital sulcus, near to the mid-line (their designation 3 and 4) and also at the lateral posterior portion of the sulcus. These areas correspond roughly with our designations of *B* and *F*; the fore-leg component in the lateral area, *F*, was found by us to be small and Gray and Turner record extension and flexion of the fingers only. But quite close to this lateral area, Gray and Turner

placed a center (no. 9) which gave on stimulation movements of the tongue; and they also designate this area as part of the wide area having control of movements of the vibrissae. Anterior to the orbital sulcus and somewhat more laterally than we would place the center for facial movements, Gray and Turner located areas for the orbicularis oculi and for movements of the snout with superimposed movements of vibrissae. All of these movements in our more general grouping would fall into the class of facial-masticatory contractions and the correspondence of areas would roughly be identical. With the very wide distribution of an area for movements of the vibrissae as described by Gray and Turner the findings recorded in the foregoing pages do not closely coincide, except at the two ends of the wide area. Nor have we been able to demonstrate the posterior, centrally located area in the cortex (no. 7 in their designations) which on excitation caused erection of the ear. With the more general grouping of the individual movements, however, the findings in the present series of animals coincide fairly exactly with those of Gray and Turner.

The conclusion of Cunningham that a center for hind-leg movements does not exist in the cortex of the opossum is supported by the findings of this series of pouch-young and adult animals. That such a center did exist was apparently demonstrated by the otherwise splendid work of the Vogts but the findings of Rogers and of Gray and Turner and the similar observations here recorded justify the assumption that in the Virginian opossum no true center for movements of the hind-leg and of the tail does exist.

Many of the findings recorded in the foregoing pages require histological study before the full significance or the interpretation of the phenomena can be made. Such studies are in progress and will be reported within a short time.

SUMMARY

A study of the responses to faradic stimulation of the cerebral cortex in a series of pouch-young and adult opossums has been made. In the youngest opossums used (23 days old), movements of the contralateral fore-leg were readily obtained; no additional motor centers were demonstrated until the cerebral cortex of a pouch-young of 76 days of age was stimulated. These additional areas were found to be concerned with movements of the facial-masticatory muscle-complex. Similar areas for movements of the face and fore-leg were demonstrated on the adult opossum in situations coinciding closely to those of other workers. No centers yielding on stimulation movements of hind-legs or of tail were found.

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DECEREBRATE RIGIDITY IN THE OPOSSUM

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Received for publication January 10, 1925

Since Sherrington's first description (11), (12) of the prolonged contraction of the extensor muscles which follows removal of the cerebral hemispheres and basal ganglia, many workers have investigated this condition of decerebrate rigidity. Not only have attempts been made to ascertain the anatomical pathways concerned but the relation of various other components of the nervous system to the phenomenon has been studied. Likewise the metabolic activities of animals in this state have been determined. Almost every angle of the problem has been attacked in some way and in many respects unanimity of opinion prevails regarding the condition which Sherrington (14) in later publications definitely established as a postural readjustment of the organism.

The reactions of adult animals subjected to decerebration have already been adequately described for the common laboratory mammals—cat, dog, guinea pig, rabbit and monkey. But in other mammalian forms—particularly those at the lower end of the mammalian tree—there has been lacking until very recently any information regarding the occurrence of rigidity after decerebration. Rogers (9), (10) has given the only account of the reactions of adult opossums to transection of the brain stem at the level of the colliculi. In his detailed report, which appeared after this work was completed, Rogers stated that decerebrate rigidity in the opossum very closely resembled that of higher mammals, appearing in back, all four legs, and in tail. Manipulation of the joints of the legs was found to augment the extensor stiffness while noxious cutaneous stimuli inhibited it. Rectal irritation decreased or totally inhibited the rigidity and was followed by a series of perineal reflexes. The rigidity persisted without intermissions for long periods of time as in the higher mammals.

The writers had during the past year an opportunity to subject to decerebration a number of adult opossums (Didelphys virginiana) as well as a fairly large series of pouch-young of the same species. Their interests in this study had been aroused by the possibility of ascertaining the exact reactions of this marsupial after decerebration, with particular reference to the degree of rigidity developed in the hind-legs of the animal, for their

own observations, reported in a foregoing paper (17), had confirmed the view of certain other investigators that there does not exist in the cerebral cortex of this animal a motor center for movements of the hind-legs. It was believed that under these conditions the occurrence or lack of the extensor contraction in the hind-legs might be of some possible significance.

But in addition to this interest in the phenomenon of decerebrate rigidity in the adult opossum, the writers felt that a study of the pouch-young of this species after removal of the hemispheres and basal ganglia would also give information of value in the ultimate interpretation of the postural reactions. Graham Brown (4) reported in 1915 that unborn fetuses of the cat, when subjected to decerebration, showed a marked tendency toward progressive movements and did not become rigid animals like the adults of the species. In only one fetus of his series was any rigidity exhibited and in this animal the extensor phenomenon was so transitory that no stress was laid upon the observation. In all of the fetuses, however, "the procedure of decerebration was followed by well-marked and long-sustained movements of progression."

In 1917, Weed (16) reported the results of decerebration in forty kittens, of which twelve showed on appropriate excitation prolonged movements of progression. Eight of these twelve kittens were 5 days or under in age while the oldest kitten to show the prolonged progressive movements was 16 days in age. In the majority of the twelve kittens no decerebrate rigidity could be made out; the rigidity seemed to be present only in the older, less active animals in the series. An inexact reciprocal relationship between the occurrence of the prolonged progressive movements in the decerebrate kitten and the extensor rigidity was indicated.

Recently Langworthy (7) published observations of the same kind, subjecting to decerebration not only kittens but also rabbits and guinea pigs soon after birth. In this way he obtained excellent material for critical control, the rabbit being born in very immature state while the guinea pig is so well developed at birth that it can almost fend for itself. In Langworthy's experiments, twenty-two rabbits, varying in age from 1 hour to 34 days, were decerebrated: the younger preparations were subsequently tremendously active, exhibiting prolonged progressive movements which often continued for over an hour. No decerebrate rigidity was present in these animals but appeared in the older rabbits of the series which showed marked diminution in the progressive rhythms. The rigidity apparently began to develop about the time when the young rabbits were able to support their weight on their legs. In the guinea pigs, all but one of the decerebrate preparations, including several animals less than one day in age, exhibited typical phenomena of decerebrate rigidity but none of the guinea pigs showed any tendency whatsoever toward progressive movements.

These observations formed the background against which the present study of the phenomena of decerebrate rigidity in the opossum was undertaken. Not only was it desired to obtain information regarding the characteristics of the extensor stiffness in the adult but it was also felt that additional data regarding the developmental aspects of decerebrate rigidity could be had from the pouch-young of this animal. For the opossum is born very immaturely at a length of 10 to 12 mm., at a stage when the hind-legs are rudimentary buds; and in the course of the next few weeks, the pouch-young may be considered to be air-breathing embryos and fetuses. And in such experiments it may be said that satisfactory results will follow only when the animals are well adapted to the ventilation of the lungs; the premature delivery of a fetus from one of the common mammals has not in our experience afforded satisfactory material for such investigations.

METHOD OF INVESTIGATION. In practically all of the opossums, preliminary determination of the motor areas was carried out under ether anesthesia; the exposure of the cerebral cortex afforded ample opening in the skull for the procedure of decerebration. As soon as the motor reactions to electrical excitation were determined in the animal, the brain stem just anterior to the posterior colliculi was transected with a blunt spatula and the anesthesia immediately discontinued. The hemispheres and basal ganglia were then removed through the cranial opening and the bleeding controlled with cotton pledgets. The cranial cavity was subsequently loosely packed with cotton wool and the animal suspended in an appropriate fashion, or placed on a warm pad.

The procedure of decerebration in these opossums varied from that usually employed for adults (15) only in the fact that the carotid arteries were not ligated, except in one adult, as a preliminary to the transection. This ligation was found unnecessary as the whole operation could be easily carried out without undue loss of blood. The body temperature of the decerebrate animals was maintained by adjustment of electric lamps.

In all, twenty-one opossums were subjected to decerebration, the transections being made as nearly as possible in exactly the same plane. Of these animals, eight were full-grown adults while the remainder were pouch-young, varying in age from 23 to 89 days. The oldest of the pouch-young were really no longer in the pouch and may be considered as young opossums just able to care for themselves.

As soon as the anesthesia had passed off, the reactions of the animals were carefully studied particularly with regard to the onset of a definite extensor rigidity. These observations were then continued for various periods of time and the reflex responses carefully noted. At the conclusion of the study the animals were killed and the brain stem fixed in 10 per cent formalin for anatomical control of the neurological lesion.

EXPERIMENTAL RESULTS. As the phenomena exhibited by the adult animals after decerebration differed quite markedly from those shown by the pouch-young of various ages it seems desirable to detail the findings under the two headings, beginning with the adult animals and then proceeding to the pouch-young. In this way it will be possible to point out the great similarities in reaction to decerebration of the adult opossum to those of higher mammals and to show that the responses of the pouch-young to this experimental procedure have many characters in common with those of immature animals of other species.

Adult opossums. In this series, eight adult opossums, male and female, were subjected to decerebration by transection of the neuraxis, with a transverse cut which passed ventrally just anterior to the inferior colliculi. The motor cortex of all of these animals had been worked out immediately before the decerebration was performed and in consequence they had all been under the influence of ether anesthesia for periods from 50 to 120 minutes. The average duration of the period of anesthesia was 80 minutes—considerably more time than would have been required for the surgical procedures necessary for the decerebration alone. Decerebration was quickly performed at the end of this period of anesthesia and as removal of the cerebral hemispheres destroyed consciousness, no further anesthesia was employed.

Immediately after the decerebration, observations regarding the first appearance of the rigidity were made. In two of the eight animals, a definite extensor stiffness made its appearance in the elbow within 5 minutes after the transection; in the other animals it was delayed for various periods up to 2½ hours, as in one animal. Five of the preparations showed evidence of the onset of a decerebrate rigidity in the fore-legs within 20 minutes. In none of these adult animals was rigidity lacking during the period of observation.

The rigidity when first made out in the elbow joint, was quite similar to that of cats or dogs, in which the phenomenon has been accurately described. The posture of the fore-legs first gave evidence of the existence of an extensor stiffness which could be well appreciated by passive movements of the leg. Decerebrate rigidity was next found in the shoulder joint in these adult opossums, but it did not invariably involve the wrist joint, as far as the observations in this series of animals demonstrated. The rigidity caused an out-thrust of both fore-legs in such a manner that adduction seemed marked and crossing of the two legs was frequently observed.

From the fore-legs the extensor stiffness seemed to spread into the muscles of the back and of the tail. The head was usually drawn back in extension, the degree being, however, variable and in several animals not extreme. The tail exhibited usually this dorsal bowing to a far greater

extent, extreme dorsal flexion or extension being observed in over half of the animals in the series. This involvement of the tail in the extensor stiffening was frequently only in the proximal one-half, the distal portion being in these cases quite flaccid.

After the condition of decerebrate rigidity had definitely made its appearance in the musculature of the fore-leg, back and tail, a considerable period of time usually elapsed before evidence of involvement of the hind-legs in the process could be made out. In two of the eight animals, slight rigidity was noted in the muscles about the knee joint within 25 minutes; in these animals, rigidity had appeared in the fore-leg in 10 and 15 minutes after the transection. In five other animals in the group, definite evidences of decerebrate rigidity in the hind-legs was not obtained until at least one hour had elapsed; the onset was at times delayed until 3 hours had passed. In one animal in the series, at the end of 4 hours it was questionable whether any real decerebrate rigidity was present in the hind-legs though in this particular animal outspoken stiffness occurred in the fore-legs within 5 minutes after transection of the brain stem.

The extensor rigidity, when it at length appeared in the hind-legs of these opossums, spread to the muscles about the hip joint soon after it had affected those about the knee. The involvement of the hip joint was was usually not expressed in a postural reaction but was readily appreciated when passive movement of the joint met with resistance. Very rarely was there any rigidity to be made out in the ankle but occasionally a fair degree of extensor out-thrust was present in the muscles activating this joint. Taken as a whole, however, it appeared that the amount of decerebrate rigidity in the hind-legs of these opossums was never as great as in the cat, after identical experimental procedures.

Likewise, it was found that the degree of decerebrate rigidity varied greatly in these opossums throughout the periods of observation. In three of the eight animals, as soon as the anesthesia had passed off, the extensor stiffness continued without alteration or decrease for several hours until the animals were killed. In others, a marked degree of rigidity was frequently followed within a half hour by a period of greatly decreased extensor stiffness—a phenomenon for which no apparent cause could be found in the particular animal. Almost invariably in such cases the outspoken rigidity returned and persisted uninterruptedly. In other animals, the usual bilateral equality of the extensor out-thrusts was intermittently interrupted, so that there was present at times only a unilateral stiffness of variable degree. This asymmetrical condition was usually followed by resumption of the bilateral symmetrical rigidity; at times the rigidity alternated from side to side. In all of these reactions the position of the animal seemed to have but little relation to the changing character of the extensor out-thrusts.

An even more striking phenomenon of these decerebrate opossums was the frequent occurrence of rhythmic beats of all four legs, in an alternating manner, giving movements of diagonal four-footed progression. These movements occurred in five of the eight animals at some time during the period of observation; they were equally marked in those animals showing great rigidity as in those in which the rigidity was undergoing intermittent interruptions. When these progressive movements were first noted, it was thought that they might possibly be due to asphyxial stimulation of the nervous system; such a view was quickly found to be erroneous. Throughout the progressive movements respiration in these animals continued without alteration or with but slight acceleration and increase in amplitude. Removal of the loose cotton packing in the cranial cavity, to obviate any possible pressure upon the brain-stem likewise had no apparent effect upon the rhythmic beats. The movements seemed related primarily to the original ablation of a portion of the central nervous system and not to any unforseen chance occurrence during the subsequent course of the experiment. In the other three animals of the series no rhythmic movements of any character were noted.

The reflex activities of these decerebrate adult opossums were quite similar to those of the higher mammals under identical experimental conditions. The very strong stimulus of partial crushing of a paw was required for the retraction of an extremity out-thrust in the rigidity; the mild excitation of pinching of a foot pad rarely sufficed to inaugurate the withdrawal of the leg. Gentle stroking of the side of the pinna usually caused a quick movement of the ear away from the stimulus and it was noted that this reaction became accentuated during the frequent periods of progressive movements. The scratch reflex, elicited by stroking the animal at the base of the ear, was frequently obtained and showed no essential differences from the reaction in cats or dogs.

The decerebrate rigidity in these adult opossums was, as in the other mammals, of a plastic character. Postural readjustments, made by the animals without apparent external stimulation, indicated the essential plasticity of the extensor out-thrust but the plasticity could be much more clearly shown by passive alteration of the position of the animal's head. When placed upon its four feet, depression of the head in such a preparation resulted in a lowering of the shoulder region and almost no elevation of the hind quarters, while raising of the head with elevation of the nose caused practically no lowering of the hind quarters but an even greater out-thrust of the fore-legs. These reactions, both in character and in rapidity of execution, were quite similar in all essential respects to the plastic readjustments so well described in the higher mammals by Sherrington (14).

In one animal, in which the rigidity was outspoken in all four legs,

electrical excitations of the cut surface of the midbrain were undertaken. Stimulation with a mild induced current of the tegmental areas of this cut surface caused diagonal walking movements in all four legs, the hind-legs being in extension at the end of the period of excitation. Intracranial stimulation of any of the three branches of the trigeminal nerve resulted in a tremendous accentuation of the decerebrate rigidity as manifested by the extensor out-thrusts; this accentuation endured for some seconds after the cessation of the excitation and was then followed by a short period of diminished rigidity. Appropriate stimulation of the mesial fractions of the cerebral crura was followed by a diminution of the extensor out-thrust in the ipsilateral fore- and hind-legs; the reactions obtained after such stimulation were highly suggestive but not conclusive.

The results of these experiments on adult opossums, then, indicated that transection of the brain stem in the region of the colliculi was followed by the appearance of a decerebrate rigidity which in many essentials was quite similar to that of the higher mammals. The relatively slight involvement of the hind-legs in the extensor out-thrust, the frequent occurrence of bilateral movements of progression and the very marked involvement of axial musculature are phenomena which permit one to differentiate its characteristics from those of the higher mammals.

Pouch-young. When the pouch-young of various ages were subjected to the same procedure of transection of the brain stem at the level of the colliculi, there was hardly the same uniformity of subsequent reaction. In consequence it is necessary to record the findings in some detail for each individual animal in order that the many reactions noted only in a single animal may be given just weight.

The youngest animal subjected to decerebration was a pouch-young opossum (no. 18) which was 23 days old and measured 33 mm. in crown-rump length. This animal was decerebrated after 40 minutes etherization, the transection of the brain stem passing just anterior to the inferior colliculi. For a period of 40 minutes the animal remained in good physical condition and exhibited, after the anesthesia had passed off, a certain degree of reflex activity. Pinching of the skin or electrical stimulation caused rather rapid flexor withdrawals of the fore-legs but in the hind-legs no similar reactions were elicited. The animal made no spontaneous movements after decerebration nor did any rigidity appear in any of the legs.

In the next animal (no. 34), which was 41 days old and which measured 48 mm. in crown-rump length, decerebration at the same level was performed after 35 minutes of etherization. Fifteen minutes after the transection, the animal exhibited when suspended the phenomenon of intermittent prolonged progression, the fore-legs beating in vigorous alternation while the hind-legs showed only the slightest tendency toward

such rhythmic movements. The progressive movements of the fore-legs continued for various lengths of time, being usually of about one minute's duration. These movements were then followed by periods of inactivity, during which different local reactions were elicited. Electric stimulation of the foot pads of the fore-paw resulted in quick retractions of the fore-leg while similar excitation of the skin near the elbow caused an extensor out-thrust of the ipsilateral fore-leg. Stimulation of the perineal region always caused walking movements of all four legs. These reflex reactions and particularly the phenomenon of progression were studied for some time, and as the animal was still in good condition, the spinal cord in the lower thoracic region was rapidly severed with scissors. This complete section of the cord caused immediate flexion of both hind-legs, to be replaced after a few seconds by well-defined and extreme extension of the hind-legs, without intervention of any rhythmic alternating movements. At no time throughout the period of experimentation were any signs of a decerebrate rigidity observed.

In another pouch-young of 41 days' age (58 mm. crown-rump length) from a second litter, decerebration was followed by a gradual development of activity but at no time during the remainder of the experiment was there evidence of spontaneous progression. During the application of a fairly strong faradic current to the skin, typical alternating movements of the legs were observed; these continued only during the period of electrical excitation. On section of the spinal cord in the mid-thoracic region, the fore-legs moved for a short time in a progressive rhythm while the hind-legs were drawn up in extreme flexion. No extensor rigidity occurred at any time.

Two pouch-young animals 47 days of age, measuring 60 and 66 mm. in crown-rump length, were similarly subjected to decerebration. The first of the 2 animals did not become very active, apparently because of poor physical condition. Shortly after the transection this opossum made a few beating movements of the fore-legs, and later by appropriate electrical and mechanical stimulation, slow but regular rhythmic movements, lasting slightly more than thirty seconds in duration, were elicited. In the second animal, about the same degree of spontaneous activity was exhibited: a few rhythmic beats of the fore-legs with occasional interpolated movements of the hind-legs were the only evidences of this activity. In neither animal were the extensor out-thrusts of decerebrate rigidity noted.

The next animal in the series was one of 54 days' age, measuring 62 mm. in crown-rump length. Fifteen minutes after decerebration, slight pinching of the tail was followed by rhythmic movements of the legs; later these progressive movements became more frequent and prolonged, being inaugurated by very mild excitation and exhibiting the typical

alternation of four-footed progression. The general cutaneous reflexes were particularly active but at no time was there any evidence of a decerebrate rigidity, as expressed in a prolonged contraction of the extensor muscles.

A pouch-young animal of 56 days' age (measuring 74 mm. in crown-rump length) was one of the most active of this series. Within 10 minutes after decerebration, the animal became very restive and it made occasional spontaneous movements of the hind-legs. The general responsiveness of the animal increased as shown by the heightened tendency toward rhythmic alternating beats; well-executed movements of progression then followed suspension of the animal. An hour after decerebration, it was noted that the prolonged movements of progression were not perfectly coördinated, the fore- and hind-legs beating in somewhat different rhythm, but in general the animal seemed to be as active and to walk as well as in the normal state before the experimental procedures were instituted. The animal responded to auditory stimuli but at no time showed any decerebrate rigidity. On section of the spinal cord in the thoracic region, extreme flexion of both hind-legs with ventral curling of the tail at first occurred; this phenomenon was soon followed by a series of alternating beats of the hind-legs which ended abruptly in extreme extension.

The next animal experimented upon (62 days old, 82 mm. in crown-rump measurement) showed almost the same degree of spontaneous activity after decerebration, though the first evidences of this activity were not obtained until 35 minutes after the transection. From this time on the animal became increasingly active, making repeated sucking noises and hanging on to objects with his tail. One hour after the transection, it was recorded that the animal was exhibiting activities quite similar to those shown before decerebration. At rest the animal remained in a crouching position but it elevated itself upon its four feet in response to loud auditory stimuli and then carried out prolonged walking movements. These movements were fairly well coördinated but the animal was more unsteady upon its feet than before decerebration. When placed in a large glass jar, the animal walked persistently and did not cease the purposeless movements until removed from the jar. After an hour of such prolonged progression, the hind-legs were observed to be moving at a somewhat slower rhythm than were the fore-legs but the reciprocal coordination between the movements of the two hind-legs was perfect. On being placed in a deep basin of water the decerebrate animal was able to swim quite well but was unable to keep its nose and mouth above the surface. On section of the spinal cord, the initial flexion of the hind-legs was followed by typical rhythmic beats which in turn were soon succeeded by extreme extension of both legs and tail. Throughout the experiment, no evidence of any extensor rigidity was observed.

A pouch-young animal, but few days older than the one just described (64 days, 93 mm. in crown-rump length), showed after decerebration but little spontaneous activity. At times the animal reacted to pinching by making a few rhythmic movements of the legs but in general it remained unresponsive even to relatively strong excitation. No rigidity of any kind was observed in this preparation.

Slightly more spontaneous activity of a rhythmic nature was exhibited by the next animal in the series (68 days old, 96 mm. in crown-rump measurement). The opossum after decerebration was not in very good physical condition but at times it showed a rhythmic progressive beating in both fore- and hind-legs, though in general the coördination of movement between the fore- and the hind-legs was imperfect. At no time were the cutaneous reflexes easily elicited and at no time was any extensor rigidity observed.

An older opossum (82 days old, 140 mm. in crown-rump measurement) was at the stage of development when the animals leave the pouch permanently. It was able to run about actively and to climb a heavy cord without difficulty, maintaining perfect balance. After decerebration, the opossum lay for a period of an hour or more in a condition of flaccid flexion, with rather active responses to cutaneous irritation but without extensor rigidity or tendency toward spontaneous progressive movements. Seventy-five minutes after decerebration the animal developed a definite extensor stiffness in shoulder, elbow, hip and knee—a true decerebrate rigidity, though marked by flexion at wrist and ankle. During the period of this decerebrate rigidity, the tail was strongly extended over the back. This extensor out-thrust continued until the end of the experiment.

The oldest animal in this group of pouch-young (89 days old, 157 mm. in crown-rump length) was apparently quite capable of caring for itself under normal conditions. It was able to walk and run about very vigorously and could also climb a small rope with great accuracy. After decerebration, the ordinary cutaneous reflexes did not become active until 45 minutes had elapsed. At this time it was noted that the scratch reflex was easily elicited by cutaneous stimulation in the region of the base of the ear and shortly after this, the animal made spontaneous progressive movements of all four legs, no rigidity being present. The tendency to rhythmic movements of progression continued to increase and one hour after the transection, it was recorded that the animal was supporting itself on all four feet and actually walking about. Slight extensor rigidity was noted in the tail at this time but because of the continued rhythmic movements it was impossible to make out whether there was any rigidity in the legs. One hour and a half after decerebration the opossum was observed to walk with the legs more stiffly extended than normally but it was in constant motion with practically no periods of rest. An hour later the

animal's reactions were quite similar—almost incessant progression on maximally extended legs and very active responses to cutaneous excitation. Between the periods of progression, however, when the animal was at rest, it was possible to make out a definite extensor rigidity in both fore- and hind-legs, affecting especially the elbow. But throughout the whole period of experimentation the tremendous progressive activity replaced or obscured the evidences of the extensor out-thrust.

The series of pouch-young opossums showed, then, no real evidence of an extensor rigidity until a marked extra-uterine development had occurred; in the stages previous to this, the characteristic picture was that of an active progressive tendency though many variations in the individual reaction occurred. It is felt that the long period of etherization caused diminution in the subsequent activities of many of the preparations; decerebrate pouch-young in good general physical condition seemed always to be tremendously active in their responses.

Discussion. The observations regarding the occurrence of decerebrate rigidity in the adult marsupial (D. virginiana), as detailed in the foregoing sections of this report, are in general accord with the recent findings of Rogers (9), (10) in this animal. Rogers, however, considered the opossum to show practically no difference in reaction to this neuraxial transection from the well-known phenomena exhibited by the higher mammals. The present series of experiments indicates that the extensor contraction in the fore-legs of the opossum is quite similar to that of the other mammals but the involvement of the hind-legs is usually far less extreme in degree than that of the cat, dog or monkey. In the other respects the rigidity as seen in the opossum is in the main quite like that of the other mammals though the tail is usually much more strongly extended. There is a plastic quality to the rigidity quite comparable to that already established for the higher mammals. A striking difference between the reactions of the decerebrate marsupial and those of the higher mammals lies in the fact that the opossums, upon whose reactions this paper is based, did not exhibit an enduring type of extensor out-thrust but in the majority of the cases progressive movements replaced for various periods of time the rigidity which otherwise characterized the animal's reaction.

The pouch-young of various ages likewise showed marked tendencies to progressive rhythms after decerebration. In all these animals, the outstanding features were the progressive movements and except in the two oldest there were no signs of an extensor stiffness. In the latter, however, definite signs of a decerebrate rigidity were made out during the periods of quiescence between the rhythmic movements.

These findings are of interest when considered in connection with the previous work done on the subject of progressive movements in decerebrate animals. The observations of Graham Brown (4), of Weed (16) and of

Langworthy (7) are in general agreement in showing that in fetal and very young kittens progressive movements are characteristic of the decerebrate phenomena; Langworthy has furthermore demonstrated that rabbits during the first weeks after birth practically always exhibit rhythmic movements while guinea-pigs show the typical extensor stiffness of well-known adult preparations. In all of these former experiments on young animals an inexact reciprocal relationship between the occurrence of prolonged progressive movements and the extensor rigidity was indicated.

With the results of these earlier experiments, the present series of observations upon the pouch-young opossums are in complete accord. In the youngest animals in the series, no rigidity but a marked progressive tendency was characteristic; in only the 2 oldest was a real extensor rigidity observed—and here only during the quiescent period between the progressive rhythms. The inexact reciprocal relationship between the two phenomena seems therefore to be repeated in the opossum. But in addition the experiments showed that under the ordinary conditions of observation the tendency toward progressive movements was not entirely suppressed or submerged by the decerebrate rigidity in the adult opossum as in the acute preparations in higher mammals.

If the decerebrate rigidity be taken to be merely an accentuated postural contraction of those muscles resisting the force of gravity (14), the relation between posture and progression becomes at once a subject of interesting speculation. As far as can be told from this series of experiments and from those previously reported, the mechanism for rhythmic alternation of leg movements seems to be developed before the mechanism for posture. When, however, the nervous mechanism for the maintenance of posture is functionally complete in the developing animal, it suppresses the progression as judged by the development of the inevitable rigidity, following the experimental procedure of decerebration. Such a suppression or inhibition of the progressive mechanism apparently does not mean that the mechanism cannot, in response to other excitations, be brought again into action, for as Graham Brown (1) and Forbes (5) independently showed, such rhythmic alternation of movements can be effected by the balance of two antagonistic reflex stimuli. In all of the mammals therefore it seems likely that the postural mechanism becomes the dominant one following decerebration in the adult; in the young animals of different species, the progressive tendencies are outspoken if the animals are born in an immature state, or suppressed if the animals are born in a more mature state. In the growth of the immature animals in the first 2 or 3 weeks of extra-uterine life, the nervous mechanism for posture becomes developed, and when this development is almost complete the animals for a period exhibit both progressive movements and in the periods of quiescence, a true extensor rigidity. At such stages it may be assumed that

neither mechanism is dominant but that they are approximately equally balanced, so that first one and then the other mechanism gives forth its characteristic reaction. In all of the higher mammals, the nervous mechanism for posture entirely suppresses the tendencies for progression in the adults, as shown by the reactions after decerebration; but in the opossum, the dominance of the postural reaction is not as great and progressive movements of all four legs make their intermittent appearance after such experimental ablations.

The appearance of the rhythmic alternating movements in very immature animals after decerebration does not indicate necessarily that the animal possesses the power of independent progression in the normal state. For proper walking or running, the maintenance of posture is essential and most of these very immature animals lack this power if one may judge by their normal sprawling state. Likewise the lack of rigidity after decerebration in these very immature animals gives evidence of want of real postural adjustment. Such decerebrated animals, if placed on a flat surface during the period of active running or walking movements, rarely succeed in making the movements effective for progression; they are unable to maintain a proper balance or the necessary postural elevation of the body, so that they usually fall to one or other side. When, however, they develop sufficiently to maintain posture, then the rhythmic movements of alternating flexion and extension become effective and progression results.

Laughton (8) has recently reported observations regarding the location of the centers for progression in certain mammals (cat, dog and rabbit). Laughton's experiments led him to conclude that the intactness of the caudal two-thirds of the thalamus was necessary for progression in the cat and dog, while in the rabbit the cephalic two-thirds of the pontine region only was needed. The occurrence of a marked rigidity in any of these animals did not inhibit the coördinated movements of progression, which seemed to break through and become superimposed upon the rigidity. Laughton furthermore carried out similar experiments on kittens of 4 weeks of age, obtaining the same results as in adults. Kittens of this age, it may be said, are beyond the age at which decerebration is followed by progressive movements (cf. Weed (16), Langworthy (7)), and the same remark may be made of his experiments on puppies of 8 weeks of age (cf. Langworthy (7)).

Laughton's experiments delimit in a very accurate way the centers for progressive movement in these animals after removal of certain of the higher portions of the nervous system. Since the early experiments of Goltz (6) it has been well known that animals deprived of the cerebral cortex are able to execute movements of progression in satisfactory fashion. Many subsequent observers have added data to the general subject but no one has so carefully delimited the areas as has Laughton. But it is

apparent from the experiments here reported and the previous publications of Graham Brown (4), of Weed (16) and of Langworthy (7), that progression in the immature animal occurs after more extensive ablations of the nervous system than in the adult. There is, moreover, a nervous mechanism in the spinal cord itself for the coördination of movements necessary for progression as shown by many experiments of Sherrington (13) and Graham Brown (2), (3). Added to this spinal mechanism there is presumably a higher center which is essential for the type of prolonged progression described in the immature animals and in adults. In the adults a still higher center is necessary for breaking through the postural contraction, but in the immature animals the functioning of this higher center is apparently not necessary because the postural mechanism has not as yet developed.

SUMMARY

Decerebration in adult opossums was followed by the development of a true rigidity, involving the extensor musculature of neck, trunk, tail, forelegs and to a lesser extent of hind-legs. The reactions were quite similar to those of the higher mammals subjected to similar transections of the brain stem. The decerebrate adult opossum differed from the higher mammals under similar experimental conditions by exhibiting very frequently rhythmic, well coördinated movements of progression. In the pouch-young opossums, decerebration was followed, except in the two oldest of the series (82 days and 89 days old), by the occurrence of progressive movements of a prolonged nature, without an extensor rigidity. In the two oldest of the pouch-young, there was evidence of a true rigidity in the intervals of quiescence between the periods of progressive movements.

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RELATION OF GASTRIC CONTENT TO THE PHYSIOLOGY OF THE COMMON DUCT SPHINCTER

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Received for publication January 8, 1925

While working upon the visualization of the gall bladder with tetrabromphenolphthalein and other substances (1), (2), numerous attempts were made to increase the density of the shadow by modifying the technique. It is an evident fact that the production of the shadow is due primarily to the concentration of the bile containing the tetrabromphenolphthalein within the gall bladder and its retention there while being concentrated. The factor controlling this stasis of bile within the biliary system is the sphincteric action of the sphincter of Oddi. Obviously, anything which causes relaxation of this sphincter will release bile and allow the escape of bile from the biliary system and vice versa. It has been shown by Meltzer (3) and others that magnesium sulphate relaxes the sphincter and it is just as logical to assume that some method, probably chemical, can be devised to produce a contraction of the sphincter of Oddi.

It was found that the sphincteric action of the sphincter of Oddi could largely be controlled by varying the alkali and acid content of the stomach. A fact which seems not to have been observed before, although it has been known that variations in the reaction of the duodenal contents affect the sphincteric action.

Method. Under ether anesthesia, the common duct of a dog is cut as far away from the duodenum as possible. A long glass tube (about 100 cm. long and $\frac{3}{4}$ cm. in diameter) is connected with a cannula by means of a rubber tube, filled with weak solution of methylene blue and the cannula inserted into the distal end of the common duct. The pressure against the sphincter can be altered at will by raising or lowering the glass manometer. An incision 2 cm. long is made in the duodenum over the papilla of Vater, and a ligature tied around the pylorus and cardia. The contents of the stomach are removed through a small incision into which is fitted a T-tube through which the stomach can be irrigated. The point at which the fluid ceases to flow through the papilla is taken as the pressure reading. Various solutions are then drained into and out of the stomach and pressure readings are taken every minute or two. Various modifications of

this method were tried. Readings were taken without opening the duodenum or stomach and without tying off the pylorus and cardia.

Results of experiments. If the stomach of the animal contains any food, the papilla in most instances will be found to be discharging bile. When the stomach is incised and the contents removed, the bile flow will practically always cease and the pressure required to open the papilla will vary between 40 and 100 mm. of water. The readings obtained after opening the stomach and removing the contents, if any are present, are taken as normal. Any readings taken before incision of the stomach can scarcely be used for comparison because the stomach must be opened later to allow the injection and removal of the various substances. Irrigation with saline almost invariably raises the pressure 10 to 30 mm. Filling the stomach with 75 to 150 cc. of 0.5 per cent sodium hydroxide causes an immediate rise in pressure of 100 to 225 mm. Replacement of the sodium hydroxide with 75 to 150 cc. of 0.5 per cent hydrochloric acid causes an immediate drop in pressure of 75 to 150 mm. These fluctuations of pressure are usually most marked when no incision is made in the stomach and the solution is injected with a syringe and needle. Likewise, the fluctuation is usually greater when the pylorus and cardia are not tied. (Overflow into the duodenum is prevented by pressure over the pylorus or insertion of a plug into the lumen of the pylorus through an incision into the duodenum.) Application of 25 per cent magnesium sulphate to the stomach in the majority of instances causes a fall of pressure in the same manner that hydrochloric acid does, but these results are inconstant and do not always occur. Local application of alcohol likewise produces no constant effect. On a few occasions there was a definite fall of pressure of 10 to 40 mm.

Intravenous injection of various drugs in amounts equal to two or three times the proportionate doses used ordinarily in the human, including atropin, pilocarpine and physostigmine, had no effect on the pressure. Large doses of pituitrin in many cases produced a rise of 20 to 70 mm. from the normal. Mechanical irritation of the papilla including even the slight touch of a cotton sponge will produce a spasm which is visible to the naked eye and will cause a rise in pressure of 40 to 150 mm.

A striking effect on the pressure required to break through the sphincter is noted when the stomach is distended with fluid, regardless of what fluid or solution be used. Usually 700 to 800 cc. were required with these experiments on the dog. The pressure invariably rose 50 to 300 mm. Extreme care was exercised lest the duodenum or biliary tract be crowded or affected by contact with the distended stomach. This effect was so marked that it leads one to suspect it would apply in the same manner to the human stomach and be a factor in creating digestive disturbance on account of the delayed flow of bile into the duodenum.

Concentrated solution of sodium bicarbonate acts as does sodium hydroxide in producing a spasm of the papilla with rise of pressure, except that it is scarcely as great.

Remarks. The existence of muscular fibers in the papilla of Vater was first demonstrated by Oddi (4). The sphincteric action of the papilla has been measured by Oddi (5), Archibald (6) and Mann (7) and recorded in terms of millimeters of water pressure required to break through the sphincter. This required pressure varies with different workers between 200 and 675 mm. The normal pressure as found in this laboratory was even lower, rarely going over 150 mm.

That the application of dilute hydrochloric acid to the duodenal mucous membrane will stimulate the flow of bile through the papilla was demonstrated as long ago as 1880 by Rutherford (8). A lowering of pressure of from 50 to 100 mm. of water was noted by McWhorter (9) after application of 25 per cent magnesium sulphate to the duodenal mucous membrane. Auster and Crohn (10) demonstrated that bile flow may be induced by the application to the duodenal mucosa, of peptone, sodium sulphate, sodium phosphate, n/10 hydrochloric acid and bile, and sodium glycocholate.

No reference has been seen in the literature dealing with the relation of the stomach content to the physiology of the papilla of Vater, except a vague statement by Oddi that application of dilute hydrochloric acid to the duodenum and stomach causes a spasm of the sphincter. This cannot be substantiated in this laboratory. Furthermore, many workers, as stated above, have demonstrated a production of bile flow by the application of dilute hydrochloric acid to the duodenal mucous membrane.

Evidence is offered in this article to demonstrate that the action of the sphincter of Oddi is controlled by the stomach as well as the duodenum. The most striking change in tonicity of the muscle fibers is seen when the gastric mucosa is treated with $\frac{1}{2}$ per cent sodium hydroxide. The exact mechanism of this behavior cannot be accurately explained. Obviously, it must be controlled by the sympathetic nervous system or by hormonic action through the blood stream. The reactions, especially to alkali, are so immediate that it would seem better explained by a nervous mechanism.

SUMMARY

1. The tonicity of the sphincter Oddi is controlled by the gastric content as well as duodenal content.

2. The hydrogen ion concentration is an important if not the most important factor in controlling the sphincter from the gastric side, since so little effect is seen from the use of substances other than alkali or acid.

3. Distention of the stomach causes a very marked spasm of the sphincter of Oddi.

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THE GLYCOGEN CONTENT OF THE HEART, LIVER AND MUSCLES OF NORMAL AND DIABETIC DOGS

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Received for publication December 23, 1924

Minkowski found that the percentage of glycogen in the liver of the diabetic dog fell to 0.5 per cent or less regardless of the amount of dextrose ingested. However, when levulose was administered there was not a proportional increase in the excretion of sugar and the amount of glycogen stored in the liver was considerably increased. Because of the latter statement, levulose has for a long time been administered to diabetic patients with the hope that the patient could utilize this sugar although unable to utilize glucose. The rapid disappearance of glycogen in the liver of the diabetic animals has been confirmed by a number of investigators. That levulose can be utilized by the diabetic has been questioned. Cruickshank obtained results very different from those of Minkowski following the administration of levulose to depancreated dogs. It was emphasized by Cruickshank that his own animals suffered from a severe diabetes, and that sepsis was lacking. The possibility of Minkowski's dogs not being totally depancreatized was suggested. We have therefore carried out the experiments to determine whether there is an appreciable difference in the fate of levulose and glucose respectively in the organism of the diabetic and the partially diabetic animals.

METHOD. Dogs of excellent physical condition were selected for this experiment. The depancreatization was performed by means of blunt dissection with the fingers and sterile gauze. An orange stick was used for separating the pancreas from the blood vessels. Ligatures were applied to the pancreatic ducts and the larger arteries. The time required for the complete extirpation of the pancreas, including anesthetization and bandaging, is from twenty to forty minutes depending on the proximity of the pancreas to the duodenum. The skin was scrubbed with soap and water, painted with iodine and later rinsed with alcohol. Zinc oxide in vaseline was used as a dressing for the wounds. Suppuration was not a complicating feature of our experiments. The dogs subjected to the above experiments often lived as long as six weeks and seldom less than three weeks.

The dogs were all weighed and fed a certain amount of meat and sugar per kilogram body weight per day. The weighed diet was administered over a period of from four to ten days so as to obtain results as nearly uniform as possible. At the end of the experiment each animal was anesthetized very quickly and the heart and the liver removed. A portion of the hamstring muscles was used for the determination of the glycogen content of the muscle. Ten minutes was the maximum time used for anesthetization and the removal of the various tissues. The first tissue was being treated while the second organ was being extirpated.

The glycogen determinations were all made by one of us (R. W. L.). The estimation of the glycogen was carried out by Pflügers modification of the Kulz method as follows: 50 grams of the tissue, which had been quickly washed free of the blood with cold water and then passed through a meat grinder, were treated with 50 cc. of 60 to 70 per cent KOH solution and the mixture heated on the water bath for 24 hours. The cooled mixture was then diluted with 100 cc. of water and the glycogen precipitated by the addition of 400 cc. of 96 per cent alcohol. The glycogen was then filtered off as rapidly as possible, washed several times with 66 per cent alcohol, redissolved in a small amount of water and carefully neutralized with acetic acid. Then 5 cc. of concentrated hydrochloric acid were added, the solution diluted to 85 cc. and heated on a water bath until the hydrolysis was complete. The time required for this was from three to four hours. The glucose solution was then carefully neutralized with KOH solution, diluted to exactly 100 cc. and the glucose determination made on aliquot portions of the solution by the Hartman-Shaffer method.

Twenty-five cubic centimeters of Fehling's solution no. 1 and an equal amount of Fehling's solution no. 2 were mixed in a 250 cc. flask and enough of the glucose solution to contain from 20 to 200 mgm. of the sugar was added. The solution was made up to 100 cc., heated to boiling in four minutes, boiled for exactly two minutes and cooled under running water. The cooled solution was treated with 6 grams of solid potassium iodide and 25 cc. of 5N sulphuric acid and titrated with a N/10 solution of sodium thiosulphate. Near the end point a small amount of starch solution was added as indicator. Blanks were run on the reagents and the difference in the number of cubic centimeters of N/10 sodium thiosulphate required in the two cases, multiplied by 6.36 is the number of milligrams of copper reduced by the glucose. The amount of glucose equivalent to this weight of copper was found by reference to the Munson-Walker tables as given by Hartman and Shaffer (2).

From tables 1 and 3 we observe the glycogen content of the hearts of normal and diabetic dogs starved for 5 days to be approximately the same. Macleod and Prendergast found in two normal dogs that the glycogen in the ventricle is increased by starvation. We too find an increase in the

early days of starvation of normal dogs as well as in diabetic dogs. Thus in table 2 the glycogen content of the hearts of two diabetic dogs starved for 4 days has not fallen below normal. In tables 1 and 3 the average figures for normal and diabetic dogs starved for 5 days are 0.28 and 0.18 respectively.

The liver is the first organ to lose its glycogen in starvation in both the normal and diabetic animal. It is noticed that the disappearance of the glycogen from the liver is more rapid in the case of the diabetic than in the

TABLE 1

Glycogen in normal dogs starved for 5 days

NUMBER	HEART	LIVER	MUSCLE
1	0.31	0.43	0.1
2	0.25	0.09	0.09
Average.....	0.28	0.26	0.09

TABLE 2

Glycogen in diabetic dogs starved 4 days

NUMBER	HEART	LIVER	MUSCLE
3	0.488	0.06	0.106
4	0.48	0.08	0.21
Average.....	0.48	0.07	0.158

TABLE 3

Glycogen in diabetic dogs starved 5 days

NUMBER	HEART	LIVER	MUSCLE
5	0.23	0.05	0.03
6	0.19	0.024	0.058
7	0.12	0.06	0.05
Average.....	0.18	0.045	0.046

normal animal. The loss in the amount of glycogen is rapid at first but the glycogen left after a few days' starvation is given up very slowly. The muscle maintains its quantity of glycogen longer probably because it retains the power of storing sugar in the presence of the hyperglycemia created by the glycogenolysis in the liver.

Tables 4 and 5 show clearly the difference in glycogen content of the tissues of normal and diabetic animals when all are receiving the same diet. The most striking thing is the percentage glycogen in the heart and liver tissue of the normal and diabetic animals. The average glycogen content

of the heart muscle of normal dogs is 0.495 as compared with the diabetic heart value of 0.8 per cent. The average for the livers of normal dogs is 1.81 as compared with 0.11 in the diabetic animals.

In table 6 we see that the livers of dogs 15 and 16 contained 1.52 and 2.99 per cent glycogen when given 2 to 4 grams of glucose per pound body weight respectively in addition to 13 grams of meat per pound body weight.

TABLE 4

Normal animals fed 13 grams of lean meat per pound body-weight per day for five days

NUMBER	WEIGHT OF DOG	HEART	LIVER	MUSCLE
	pounds			
8	19	0.474	2.73	0.808
9	16	0.49	1.92	0.45
10	15	0.52	0.77	0.48
Average.....		0.495	1.81	0.579

TABLE 5

Diabetic animals fed 13 grams of lean meat per pound body-weight per day

NUMBER	WEIGHT OF DOG	HEART	LIVER	MUSCLE	REMARKS
	pounds				
11	25	0.63	0.16	0.14	Fed for 5 days
12	27	1.10	0.11	0.09	Fed for 5 days
13	20	0.52	0.06	0.64	Fed for 6 days
14	19	0.94	0.10	0.37	Fed for 6 days
Average.....		0.8	0.11	0.31	

TABLE 6

Dogs receiving 13 grams of meat and 2 grams of carbohydrate per pound body-weight per day

NUMBER	WEIGHT	CONDITION	HEART	LIVER	MUSCLE	REMARKS
15	12	Normal	0.5	1.52	0.71	2 grams glucose for 5 days
16	16	Normal	0.37	2.99	1.04	4 grams glucose for 5 days
17	41	Normal	0.46	4.63	0.57	2 grams levulose for 2 days
18	19	Diabetic	1.09	0.045	0.26	2 grams glucose for 13 days
19	30	Diabetic	0.52	0.048	0.16	2 grams levulose for 2 days
20	14	Diabetic	0.93	0.05	0.28	2 grams levulose for 4 days

Dog 19 received a similar amount of meat but 2 grams of levulose per pound instead of glucose. In this case the liver contained 4.63 per cent glycogen. The glycogen contents of the livers of dogs 18, 19 and 20 are in marked contrast to the first three of table 6. Glucose was given to dog 18 and

levulose to dogs 19 and 20, but the glycogen content of the liver and muscle tissue of these three dogs was almost identical. The average for the three livers of three diabetic dogs starved 5 days is 0.045 according to table 3. This shows very definitely that the tissue of a diabetic animal cannot store levulose as glycogen any better than glucose. The average value for the glycogen of the muscle tissue of the diabetic dogs 18, 19 and 20

TABLE 7

Dogs receiving insulin while on the diets listed below

NUMBER	WEIGHT	CONDITION	HEART	LIVER	MUSCLE	REMARKS
21	35	Diabetic 141 days	0.71	5.64	0.58	Meat plus bread
22	24	Diabetic 114 days	0.41	2.49	0.49	Meat plus bread
23	17	Normal	0.57	0.53	0.69	13 grams meat per pound per day. Convulsions for 6 hours before death
24	35	Diabetic 13 days	0.27	3.32	0.99	13 grams meat plus 2 grams glucose for 5 days
25		Diabetic 13 days	0.34	7.52	0.44	13 grams meat plus 2 grams levulose 10 days

TABLE 8

Dogs partially depancreatized and fed 13 grams meat plus 2 grams carbohydrate per pound body-weight per day

NUMBER	WEIGHT	CONDITION	HEART	LIVER	MUSCLE	REMARKS
26	17	Diabetic 8 days	0.43	0.09	0.08	2 grams levulose for 7 days, 915 mgm. pancreas tissue at autopsy.
27	31	Diabetic 9 days	0.43	2.94	0.53	2 grams levulose for 4 days, 3.7 grams pancreas tissue at autopsy
28	27	Diabetic 14 days	1.19	0.52	0.50	2 grams levulose + meat, 920 mgm. pancreas tissue at autopsy.
29	13	Diabetic 10 days	1.21	0.04	0.31	Levulose plus meat for 8 days, 375 mgm. pancreas tissue at autopsy.

Pancreas tissue is the amount left at operation and recovered at autopsy.

is 0.23. For the 3 normal dogs of table 6 the average is 0.77 per cent. The average for the muscle tissue of 3 diabetic dogs starved 5 days is 0.046. The average of the glycogen values of 4 diabetic dogs receiving meat for 5 days is 0.31. By analyzing the averages in the 4 series we find that the muscle gives up its glycogen less readily than does the liver or else retains its power of storing some glycogen for several days following pancreatectomy when large amounts of sugar are present in the blood.

Table 7 shows the results of administering insulin with a diet similar to that given to the animals in table 6. The glycogen content of the heart, liver and muscle tissue of the dogs receiving insulin was almost identical with that found in normal dogs. That is, glycogen was stored in the liver and muscle while the percentage in the hearts was 0.43 as compared with 0.8 per cent in hearts of diabetic dogs not receiving insulin.

In the series of 29 dogs four had a percentage of glycogen greater than one in the heart muscle. Dog 12 had a value of 1.10 on a meat diet, dog 18 a value of 1.09 on a diet of meat and glucose. The last two cases were those of dogs 28 and 29 with 1.19 and 1.21 per cent respectively. In dog 28 was found 920 mgm. of pancreas tissue which had been purposely left intact at the time of pancreatectomy. The pancreas tissue contained some islets and appeared normal. The liver and muscle in this case contained more glycogen than a dog totally depancreatized. Dog 29 did not store more sugar in the liver and muscle than did a complete diabetic and there was found at autopsy 375 mgm. of normal tissue. Probably these amounts of pancreas are not sufficient to exert an appreciable effect on the storage of glycogen, but we cannot imagine how Minkowski would have overlooked pieces of pancreas tissue larger than the ones we intentionally omitted. This leaves Minkowski's results of increased glycogen formation, following the administration of levulose, unexplained. It might be mentioned that the livers in the diabetic dogs receiving levulose plus meat plus insulin and the normal dogs receiving levulose contained more glycogen than the diabetic dogs receiving glucose, meat and insulin and the normal dogs receiving glucose plus meat. It may be that levulose is stored more efficiently in the presence of the internal secretion of the pancreas than is glucose. However, if such is the case it is at best only a temporary phenomenon and does not warrant the statement that levulose is utilized by the diabetic patient and is the specific sugar in diabetes.

By comparing the animals receiving the various diets, we find that the glycogen values of the hearts of all the diabetic animals receiving meat, or meat plus glucose or meat plus levulose, fell within the range of diabetic hearts. The hearts of the normal animals receiving meat, meat plus glucose, meat plus levulose, and the diabetic animals receiving similar diets with insulin, all possessed an amount of glycogen as found in normal animals. Thus taking an average of the diabetic heart values and the normal heart values, we find the average per cent of glycogen in 12 normal hearts is 0.44 with minimum and maximum values of 0.27 and 0.57 respectively. The average for 13 diabetic hearts is 0.79 with a minimum of 0.48 per cent and 1.21 per cent for the maximum.

CONCLUSIONS

1. The observations of other workers are confirmed in that there is an almost complete disappearance of glycogen from the liver following complete extirpation of the pancreas.

2. Cruickshank's work is confirmed to the effect that levulose is not stored in totally depancreatized dogs.

3. Where a gram of pancreas or less is present there is some storage of levulose but probably not more than would be stored when glucose is similarly given.

4. The average glycogen per cent of heart muscle in 12 normal dogs was found to be 0.44 per cent. In 13 totally depancreatized dogs the average value was 0.79 per cent.

5. The amount of glycogen stored in the heart, liver and muscle tissue of normal dogs is almost constant for each tissue on a similar diet. This is also true for totally depancreatized dogs not receiving insulin.

6. When diabetic dogs are given insulin the glycogen values approach those of the corresponding tissues of normal dogs.

7. Normal dogs receiving excessive doses of insulin have a markedly decreased supply of glycogen.

We wish to acknowledge our indebtedness to the Eli Lilly Company for their coöperation in supplying the Iletin for these experiments.

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FACTORS INFLUENCING THE KNEE-JERK

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Received for publication January 9, 1925

The fact that the knee-jerk is made use of so extensively as a diagnostic indication in certain cord lesions and as an indicator of the general tone of the nervous system, the writer feels justified in reporting a number of observations brought to his attention while making a rather detailed, quantitative study of this phenomenon. Reënforcement of the knee-jerk has been studied by a number of investigators. The data reported indicate that the spinal cord of a waking subject is never in a state of rest or steady activity but is subject to an ever changing ebb and flow of impulses, all of which exert an influence on the knee-jerk. The source of the stimuli that cause the impulses which increase or decrease the tone as exhibited by the knee-jerk seems to be the constant environmental changes to which all individuals are subject, as well as all changes of activity in the organism itself, both voluntary and involuntary.

The influences of the following conditions on the knee-jerk have recently been observed by the writer:

1. The effect of the force of the stimulus.
2. The effect of changing the point of impingement of the stimulus.
3. The effect of the so-called "sleep" phenomenon.

Data were collected from normal subjects in good health by means of an especially constructed apparatus (1). The apparatus automatically delivers uniform stimuli to the desired point at a constant rate. A record is made by attaching a string to the heel of a subject's shoe and thence to a wheel on an axle. A second piece of string runs from the axle of the wheel through guides to a stylus, suspended from a spring, which writes on a smoked drum. The wheel and axle serve to reduce the extent of the excursion of the stylus so that the record of any subject, regardless of the extent of his knee-jerk, can be recorded on an ordinary kymograph drum. The forward component of the excursion of the heel is used as the index of the knee-jerk. This is indicated by the distance through which the stylus moves and is referred to as the "height." In these experiments the stimuli were delivered to the right knee at the rate of seven per minute.

1. THE EFFECT OF THE FORCE OF THE STIMULUS. Lombard (2) observed that by varying the force of the stimuli used in eliciting the knee-jerk,

the height of the jerk was varied. The experiments reported in this paper not only confirm this fact but bring out other interesting results.

In this experiment seven blows of different strength were used to elicit the jerk and in all cases the lightest blow was a subminimal stimulus. They were gradually increased, thus approaching a strength which was maximal, that is, one which failed to increase the extent of the jerk.

In order to vary the force of each blow used, points 5 mm. apart were laid off on the quadrant of the tripping device (1). The strength was then changed by varying the position of the tripping lug in the slot in the quadrant. The force for each position was determined by allowing the hammer to fall against a lever hinged to the floor so that it was comparable to the leg of a subject. The lever was attached to the stylus in the same manner as the heel of the subject. By finding the force in grams required to

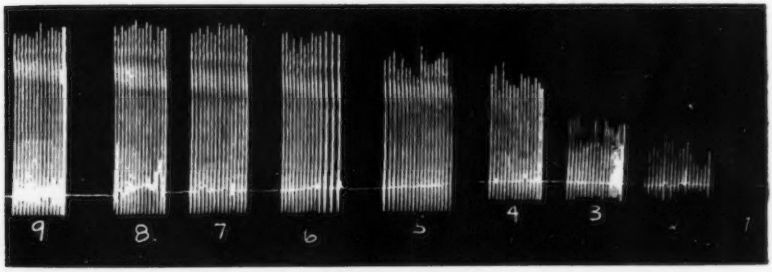

Fig. 1. *1*, 10.71 grams; *2*, 14.28 grams; *3*, 32.13 grams; *4*, 49.98 grams; *5*, 64.26 grams; *6*, 75.68 grams; *7*, 110.67 grams; (*8* and *9* were not determined since they were above maximal).

pull the stylus through a distance of 1 cm. and comparing this with the distance the stylus moved when struck by the hammer in the various positions, a value was assigned to each strength of blow used as a stimulus. The following values were found to represent the seven strengths used:

Quadrant setting	1.	10.71 grams
	2.	14.28 grams
	3.	32.13 grams
	4.	49.98 grams
	5.	64.26 grams
	6.	75.68 grams
	7.	110.67 grams

In all cases 10.71 grams was a submaximal stimulus while 110.67 grams was found sufficient to make the subject uncomfortable.

Six subjects were used in this experiment. The method of procedure was to allow the subject to write a normal record for ten minutes with the various strengths of stimuli as described above. The conditions were kept as constant as is possible in a knee-jerk experiment.

Figure 1 is representative of records obtained in this experiment. In this case 110.67 grams was a maximal stimulus.

Table 1 shows the results obtained from the subjects used in this experiment. The values presented in the table represent the average height kicked by the various subjects in ten minutes.

Conclusions. The records from all subjects studied show that an increase in the strength of the stimulus causes an increase in the height of the knee-jerk.

There is no mathematical relation between the strength of the stimulus and the increase in the height of the knee-jerk, that is, if the strength of the stimulus is doubled, the height of the knee-jerk is increased but not doubled.

TABLE 1

FORCE NUMBER	FORCE OF STIMULI	HEIGHT OF KICK					
		Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
	grams	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
1	10.71	0	0	0	0	0	0
2	14.28	0	0	1.40	0	0	10.72
3	32.13	12.20	9.38	28.18	0	0	18.33
4	49.98	20.94	27.45	49.50	0	0	35.00
5	64.26	32.86	38.00	53.46	3.92	2.48	41.28
6	75.68	38.11	48.28	55.27	17.42	3.27	52.00
7	110.67	50.93	55.92	57.75	32.15	9.31	55.46
8							55.46
9							55.33

The increase in the height of the knee-jerk due to an increase in the strength of the stimulus is greater at first but gets less and less as the force of the stimulus becomes greater and greater.

The stimulus finally becomes maximal, that is, it finally reaches a point at which the height of the jerk fails to increase. In case of the subject used in making the tracing shown in figure 1, the maximal stimulus was found to be 110.67 grams. In order to make sure of this point two stimuli of greater force were used (*8* and *9*, fig. 1). No increase in the height of the jerk resulted (subject 6, table 1). Although the data in table 1 fail to show that a maximal stimulus was reached for all subjects, the difference in height is becoming less and less as the strength of the stimuli increases.

The threshold is different for each subject studied. For subjects 1 and 2 the threshold lies between 14.28 grams and 32.13 grams; for subjects 3 and 6, between 10.71 grams and 14.28 grams; and for subjects 4 and 5, between 49.98 grams and 64.26 grams.

As the strength of the stimulus becomes greater and greater, the variation in the height of the jerks become less and less until finally, when the

stimulus is maximal, the height of the kicks approaches equality (fig. 1). It seems reasonable to suppose that when a maximal stimulus is used, the reënforcing factors are merged, due to the fact that the response is already maximal, and any additional reënforcement adds nothing to the height of the jerk.

2. THE EFFECT OF CHANGING THE POINT OF IMPINGEMENT OF THE STIMULUS. Six subjects were used in this experiment, the general method of procedure being the same as that described in part 1. The following positions were considered and will be referred to by letter in the presentation of the data and conclusions.

A. The medial edge of the ligamentum patellae.
B. The lateral edge of the ligamentum patellae.
C. The center of the patella.
D. The inferior margin of the patella.
E. The center of the ligamentum patellae.
F. The inferior end of the ligamentum patellae.
G.[1] The rectus femoris tendon.

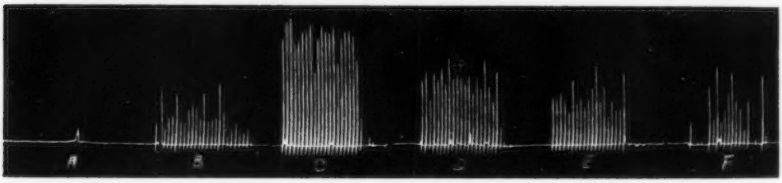

Fig. 2. *A*, medial edge of the ligamentum patellae; *B*, lateral edge of ligamentum patellae; *C*, patella; *D*, inferior margin of patella; *E*, center of the ligamentum patellae; *F*, inferior end of the ligamentum patellae.

The stimulus used in this experiment was 49.98 grams which, in the experience of the writer, is submaximal for the majority of subjects. Figure 2 is a representative tracing of the group studied.

Table 2 shows the results obtained in this experiment. The values presented in the table represent the average height kicked by the various subjects, in ten minutes.

In order to present the data given in table 2 more clearly, table 3 has been constructed.

Conclusions. Figure 1 shows that when the stimuli were applied to position A, no jerk was elicited; the highest group of kicks were recorded when the impingement was directly on the patella, while the height of the kicks in the other positions fall in the order of D, E, F and B.

[1] The apparatus used in this experiment is so constructed that it will not deliver stimuli to the rectus femoris tendon. The knee-jerk was elicited by striking the tendon with the edge of the hand.

The data in table 2 as analyzed in table 3 show that the highest jerks are not elicited from any given position. For subjects 1, 2 and 4, the highest jerks were obtained when position D was stimulated; for subject 3, position E; for subject 5, position F and for subject 6, position C.

Since positions C, D and E are not found below group 3, except in one case, and since A, B and F are not found above group 4, table 3, one is justified in concluding that the highest jerk is most likely to be elicited when the stimulus is applied to either the patella itself, the inferior margin of the patella or the center of the ligamentum patellae.

TABLE 2

POSITION	POSITION OF STIMULI	HEIGHT OF KICK					
		Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6
		mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
A	Medial edge of ligamentum patellae	5.38	15.52	0	0	2.00	0
B	Lateral edge of ligamentum patellae	20.80	21.31	0	0	0	5.04
C	Center of patella	22.46	38.95	41.80	0	6.08	34.00
D	Inferior margin of the patella	27.80	44.08	40.96	0	8.17	19.38
E	Center of the ligamentum patellae	24.69	27.45	49.50	2.61	9.31	15.31
F	Inferior end of the ligamentum patellae	19.19	26.88	37.68	0	9.72	8.85

TABLE 3

GROUP	SUBJECT 1	SUBJECT 2	SUBJECT 3	SUBJECT 4	SUBJECT 5	SUBJECT 6
1	D	D	E	D	F	C
2	E	C	C	No kick	D	D
3	C	E	D	No kick	D	E
4	B	F	F	No kick	C	F
5	F	B	No kick	No kick	A	B
6	A	A	No kick	No kick	B	A

Group 1 represents the highest kicks; group 2, the next highest, etc.

It is interesting to note that in one of the six cases, the highest jerk was recorded when the stimuli were applied to the patella itself.

The writer observed that the jerk could be elicited in two of the subjects by stimulating the rectus femoris tendon close to the superior margin of the patella. Insofar as I was able to judge, the extent of the jerk when this position was used was equally as great as when any of the other positions studied were stimulated. However, quantitative data were not collected on this point on account of the lack of apparatus for eliciting the jerk from this position.

3. THE EFFECT OF THE "SLEEP" PHENOMENON. During the course of an experiment on the knee-jerk, one subject reported that the leg and thigh had gone to "sleep." The effect was so outstanding that it seems worthy of report and is shown in figure 3.

Figure 3 shows that in this case the "sleep" phenomenon is accompanied by a depression of the knee-jerk. The jerk during the normal period (A–B), averaged 10 mm. in height, while during the "sleep" period the average was only 3 mm. This condition approaches the condition which occurs during normal sleep as shown by the writer elsewhere (3).

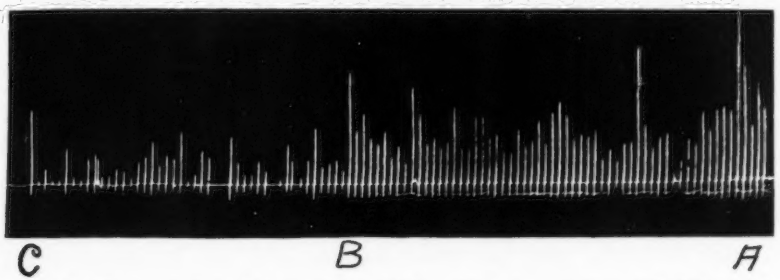

Fig. 3. This record reads from right to left. *A–B*, normal period; *B–C*, leg and thigh "asleep."

SUMMARY

An increase in the strength of the stimulus increases the height of the knee-jerk until finally a maximal response is given. The variation in the tone wave as exhibited by the jerk becomes less and less as the stimulus is increased, thus approaching a straight line.

The extent of the knee-jerk varies with the point of application of the stimulus, although there is no one position which can be assigned as the point where the highest jerks are obtained for all subjects.

When the leg and thigh are "asleep" the condition of their tone approaches that of normal sleep.

If a quantitative study of the knee-jerk is made it is necessary to explore the various positions carefully with a submaximal stimulus. In fact, it would seem that one just above the minimal should be used in order not to submerge the factors of reënforcement.

These data also indicate that if the knee-jerk is to be used as a diagnostic indicator in certain cord lesions or as an indicator of the condition of the tone of the nervous system, it is necessary to explore carefully the various areas from whence the jerk may be elicited with a uniform stimulus of submaximal strength.

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HEAT REGULATION IN THE THYROIDECTOMIZED SHEEP AND GOAT

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Received for publication December 16, 1924

It is well known that in mammals hypothyroidism results in a subnormal body temperature and it has been demonstrated that heat regulation in these animals is imperfect. For a number of years Dr. Sutherland Simpson has maintained a flock of sheep and goats consisting mainly of twins of the same sex, one twin of each pair having been thyroidectomized. In studying the effect of thyroidectomy on growth and development he has recorded the body weight and rectal temperature of each animal at regular intervals. Although the temperatures of the thyroidectomized animals were found to be, in general, subnormal, the temperature difference was not striking (1).

The present investigation was begun in an attempt to gain information concerning heat regulation in these animals following extirpation of the thyroid glands. The sheep is an unsatisfactory animal for such an experiment. It is timid and easily excited, and its coat of wool provides an insulating layer which interferes to a considerable degree with the loss of heat from the skin. Furthermore, this insulating layer is of unequal thickness in the normal and thyroidectomized animals. For example, in twins a year old, one of which had been thyroidectomized at the age of three months, while the weight of the normal animal was 88 pounds and that of the thyroidectomized sheep 85 pounds the fleece weights at shearing were 10.12 pounds and 4.50 pounds respectively (2).

Both sheep and goat possess, however, certain advantages. Complete extirpation of the thyroid glands can be performed without disturbing the external parathyroids. Experiments from Boldyreff's laboratory (3), (4) concerning heat regulation were mostly performed on thyro-parathyroidectomized dogs and cats in which tetany was imminent, the animals surviving at most but a few months. Two of the cretinoid sheep in our experiment were three years and five months old. We are thus able to take account of the factor of age in estimating the influence of thyroidectomy on temperature regulation and since the thyroid glands were extirpated

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at various periods after birth it is possible to study animals exhibiting varying degrees of hypothyroidism.

Twelve animals were employed in the present investigation, ten sheep and two goats. Information concerning them is summarized in table 1.

TABLE 1

Animals designated by the same number or letter are twins

2 op. thyroidectomized at 6 weeks 2 control 5 op. thyroidectomized at 6 weeks 5 control	Sheep 3 years 5 months of age
1 op. thyroidectomized at 3 months 1 control C op. (wether) thyroidectomized at 3 months C control (wether)	Sheep 2 years 5 months of age
3 op. thyroidectomized at 20 days 2 control	Goats* 1 year 7 months of age
16 op. thyroidectomized at 6 weeks 4 control	Lambs 5 months of age

* These goats are the surviving members of triplets (5).

TABLE 2

ANIMAL	BODY WEIGHT SEPTEMBER 22, 1924	WEIGHT OF WOOL MAY 12, 1924
	pounds	*pounds*
2 op.	71	3.0
2 control	161	9.0
5 op.	100	4.5
5 control	180	13.5
1 op.	69	2.0
1 control	132	12.0
C op.	121	4.0
C control	180	12.0
16 op.	30	
4 control	77	
Goat 3 op.	32½	
Goat 2 control	54	

Over a period of five days, from October 2 to October 7, 1924, temperatures were recorded every three hours, the readings requiring one hour. The animals were confined, a pair in each pen, in an open barn. The pens were large enough to permit them to turn about and to lie down comfortably. The vaginal temperatures of the females were recorded and the

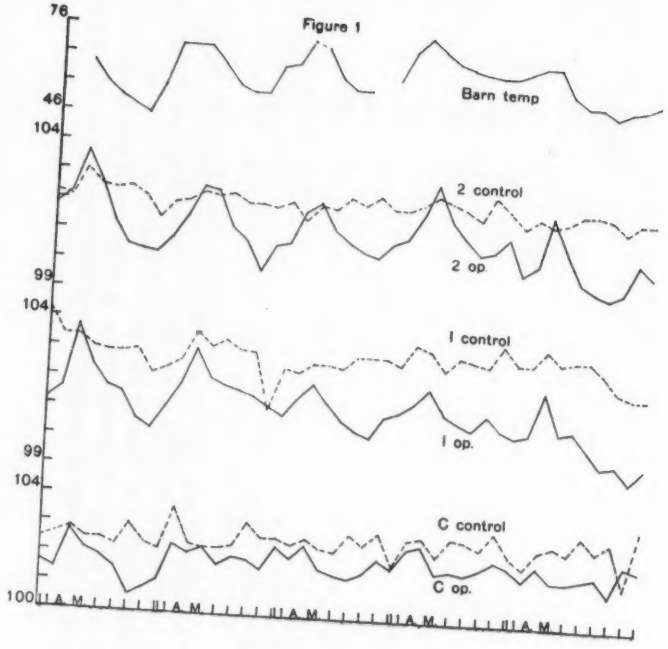


Figure 1
76
46
Barn temp
104
2 control
2 op.
99
104
1 control
1 op.
99
104
C control
C op.
100
A.M.

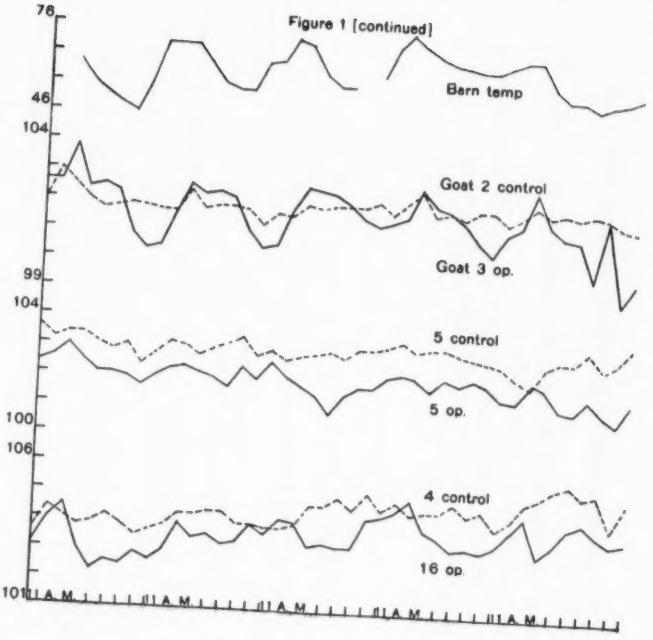


Figure 1 [continued]
76
46
Barn temp
104
Goat 2 control
Goat 3 op.
99
104
5 control
5 op.
100
106
4 control
16 op.
101
A.M.

rectal temperatures of the two wethers. The results of these observations are presented graphically in figure 1. At the top of the chart the temperature of the barn is traced and below, the body temperatures of the pairs of animals, the graphs of each pair being superimposed. All temperatures are recorded in degrees Fahrenheit. For an adequate interpretation of the results data are necessary concerning body weight and

TABLE 3

Mean body temperature and range from October 2 to October 7, 1924

ANIMAL	MEAN TEMPERATURE	TEMPERATURE RANGE
2 op.	101.3	99.4–103.6 (4.2)
2 control	102.2	101.5–103 (1.5)
5 op.	101.9	100.9–103 (2.1)
5 control	102.9	102.0–103.6 (1.6)
1 op.	101.1	99.1–103.7 (4.6)
1 control	102.9	102.0–104.2 (2.2)
C op.	101.8	100.6–102.7 (2.1)
C control	102.6	101.5–103.6 (2.1)
16 op.	103.6	102.3–105 (2.7)
4 control	104.5	103.5–105.7 (2.2)
Goat 3 op.	101.6	99.0–103.8 (4.8)
Goat 2 control	101.9	101.3–103 (1.7)

TABLE 4

Mean body temperature and range of 8 twin female lambs 7 months old one of each pair designated by "op," having been thyroidectomized when 6 weeks of age. Observations from December 6 to December 19, 1921

ANIMAL	MEAN TEMPERATURE	TEMPERATURE RANGE
1 op.	102.4	101.9–102.7 (0.8)
1 control	102.2	101.3–103.3 (2.0)
2 op.	101.8	101.2–102.4 (1.2)
2 control	102.7	101.9–104.3 (2.4)
4 op.	102.8	102.1–103.7 (1.6)
4 control	102.8	101.9–103.7 (1.8)
5 op.	102.4	101.9–103.1 (1.2)
5 control	102.5	101.7–103.6 (1.9)

fleece weight (in the case of the sheep). These data are to be found in table 2. In table 3 is given the mean body temperature together with the range of temperature for each animal during the experiment.

Discussion of results. An examination of figure 1 and table 3 shows for three of the thyroidectomized animals, viz., sheep 1 op., 2 op. and goat 3 op., a marked inability to regulate the body temperature as compared with the more adequate heat regulation of their controls. In sheep 1 op.

particularly, the body temperature curve closely parallels that of the temperature of the air and fluctuates through a wide range. In fact, a comparison of the range of variation between each thyroidectomized animal and its control (table 3) shows the degree of imperfection in heat regulation under the conditions of the experiment.

Among the animals selected for our five day experiment two were lambs seven months of age. Unfortunately, the control was found, at the end of the experiment, to be infested with the intestinal parasites. This may account for its high body temperature near the end of the period of observation. Three years previously, eight twin female lambs, one of each pair having been thyroidectomized at the age of six seeks, were confined in a small shed for thirteen days and vaginal temperatures were recorded daily at 4 p.m. for six days and at midnight for the remaining seven days. Four of these animals, viz., 2 control, 2 op., 5 control and 5 op., were employed in the present investigation. Table 4 gives the mean body temperature and temperature range for each of the eight lambs during the thirteen day period of observation.

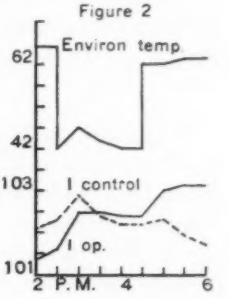
A comparison of the mean body temperatures of sheep 2 control and 2 op. in 1921 with the mean temperatures for the present experiment shows a decrease of 0.50° both for the cretin and her control. When the ranges for the animals during the early observations are compared with those obtained during the recent observations a striking difference appears. The temperature range for the control has decreased 0.90° while that for her cretin sister has increased 3° (from 1.2° to 4.2°). The heat regulating power of the thyroidectomized animal has decreased with age.

If sheep 1 control and 1 op. are compared with 2 control and 2 op. as to the mean body temperature and heat regulation little difference is apparent although the animals differ in age by a year (table 1). Both cretins show defective temperature regulation to about an equal degree.

Sheep 2 op. and 5 op. were thyroidectomized at six weeks of age while from C op. and 1 op. the thyroids were removed at the age of three months. Between 2 op. and 1 op., as mentioned above, no great difference can be found. C op., however, shows fairly adequate heat regulation although its body temperature is consistently below that of its control. This is to be correlated with its growth curve following thyroidectomy. Only during its second year has it shown distinct signs of hypothyroidism and its body weight (table 2) would indicate much less stunting than either 1 op. of the same age or 2 op. and 5 op. a year older. Details concerning the effect on growth and development of early and late thyroidectomy in sheep are to be found in recent papers by Dr. Sutherland Simpson (2), (5).

Previous to the five-day observation period sheep 5 op. had received by subcutaneous injection 12 mgm. of thyroxin. One milligram was administered every other day beginning September 8 and ending September

30, two days before temperature readings were begun another milligram was injected on October 3. After a five-day latent period neuromuscular activity as measured by a pedometer exhibited a sudden and marked increase which was maintained. This is the usual effect of thyroxin on spontaneous activity (6). If the temperature curve of 5 op. is compared with that of 2 op. of the same age a pronounced difference in heat regulating power is apparent. Although the temperature level of 5 op. remains quite constantly below that of its control it does not show the wide fluctuations with rise and fall of barn temperature exhibited by sheep 2 op., 1 op., and goat 3 op. It does, however, tend to vary with the external temperature to a greater degree than that of its control. Its heat-regulating power and body temperature level show great similarity to those of C op. This experiment on the effect of thyroxin, however, requires confirmation since temperature readings were not taken before the injections were begun.



It was of interest to determine the effect of sudden changes of external temperature on the body temperature of the thyroidectomized sheep. This was accomplished by confining sheep 1 control and 1 op. in an animal house at 66°F. for three hours and then leading them into an

TABLE 5

Heat regulation with sudden changes in the temperature of the environment

	TIME OF OBSERVATION								
	2 p.m.	2:30	3:00	3:30	4:00	4:30	5:00	5:30	6:00
Body temperature of 1 control..	102.1	102.3	102.9	102.4	102.2	102.2	102.3	101.9	101.7
Body temperature of 1 op.....	101.4	101.6	102.5	102.5	102.4	102.4	103.0	103.1	103.1
Temperature of environment....	66	66	47	44	42	42	62	63	63

At 2:30 p.m. the temperature of the environment fell from 66° to 42° and at 4:30 p.m. it rose from 42° to 62°.

anatomy vault having a temperature of 42°F. After two hours in the vault they were brought back to the animal house the temperature of which was then 62°F. Vaginal temperatures were taken every half-hour. The results are recorded in table 5 and plotted in figure 2.

It is obvious that the heat-regulating mechanism of the thyroidectomized animal responded to the sudden fall in external temperature. Its body temperature rapidly rose and continued to rise after removal from the vault to the warm room. It must be remembered that the insulating layer of wool on sheep 1 op. was much thinner that that of its control.

The temperature nerve endings would, therefore, be sooner stimulated by the cold with a resulting shivering reaction. The heat so generated being slowly dissipated would cause a continued rise of body temperature after the removal of the animal to a warmer environment. Although able to compensate for sudden temperature changes in the environment the heat-regulating mechanism did not adequately respond to gradual fluctuations of environmental temperature.

SUMMARY

1. In sheep thyroidectomized at three months or younger heat regulating power decreases with age. In the goat, also, heat regulation is rendered defective by thyroidectomy.

2. The effect of thyroidectomy on the heat regulating mechanism, as on growth and development, is more constant when the glands are removed from younger lambs than it is when the animals are older.

3. Thyroxin administered by subcutaneous injection seems to improve heat regulation in the thyroidectomized sheep.

4. The heat regulating mechanism in the thyroidectomized sheep may compensate for sudden changes in environmental temperature when it is incapable of responding adequately to gradual changes of equal magnitude.

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THE EFFECT OF THYROID THERAPY ON THE NEURO-MUSCULAR ACTIVITY OF CRETIN SHEEP

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Received for publication December 23, 1924

Recurrent periods of muscular prostration follow thyroidectomy in the sheep and goat. During these periods the animal is extremely lethargic and is almost too weak to stand. If at these times it is kept warm and dry spontaneous recovery will usually follow, but not always, the prostration often terminating fatally. Because it is desirable to prolong the life of the cretin animal, risk of death during these attacks is avoided by subcutaneous injections of thyroxin. The magnitude and suddenness of the effects of this substance are surprising. A thyroidectomized sheep or goat, which previously has been scarcely able to walk, a few days after the injections are begun is as active or more active than the normal animals in the flock. (See the moving pictures reproduced in figure 2.)

In the course of an investigation of the effect of thyroidectomy on the neuro-muscular mechanism, a method was employed for the estimation of spontaneous activity (1). By the attachment of a pedometer to the fore leg the number of steps taken by the animal can be determined. With this quantitative method available it was decided to study the effect of thyroid therapy on the neuro-muscular activity of thyroidectomized sheep. The action of thyroxin was first investigated. Tables 1 and 2 summarize the results of a number of experiments with thyroxin on cretin sheep from 5 months to 3 years 5 months of age. These animals had been thyroidectomized at 4 weeks of age and were all distinctly dwarfed at the time of treatment. In every case the thyroxin was administered by subcutaneous injection when the cretins were exhibiting great muscular weakness and lethargy. For a few days (average 5 days) no change in the animals' condition was apparent. Then quite suddenly spontaneous activity increased. In table 2 the point of sudden increase is indicated by an asterisk marking the termination of the latent period or period of no effect. The animal not only regains its lost muscular power but becomes bright and alert in appearance, in sharp contrast to its former somnolent attitude.

An interesting accompaniment of increased muscular power is seen in the state of the abdominal muscles. During periods of lethargy the

cretin exhibits meteoristic swelling of the abdomen. In fact, this swelling is one of the early signs of thyroid deficiency in the sheep and goat. After the usual latent period following thyroxin administration there is a striking

TABLE 1

Data concerning the effect of thyroxin on the activity of thyroidectomized sheep

	THYROIDECTOMIZED ANIMAL					
	4op	4op	1op	9op	11op	5op
Age at injection..	5 mo.	1 yr., 5 mo.	1 yr., 5 mo.	1 yr., 7 mo.	2 yrs., 5 mo.	3 yrs., 5 mo.
Weight at injection.....	33 lb.	55 lb.	75 lb.	57 lb.	75 lb.	100 lb.
Dosage of thyroxin.....	0.5 mgm. daily	0.25 mgm. every second day	0.25 mgm. every second day	0.5 mgm. daily	0.25 mgm. every second day	1 mgm. every second day
Latent period preceding increased activity.	8 days	3 days	6 days	6 days	4 days	4–5 days

TABLE 2

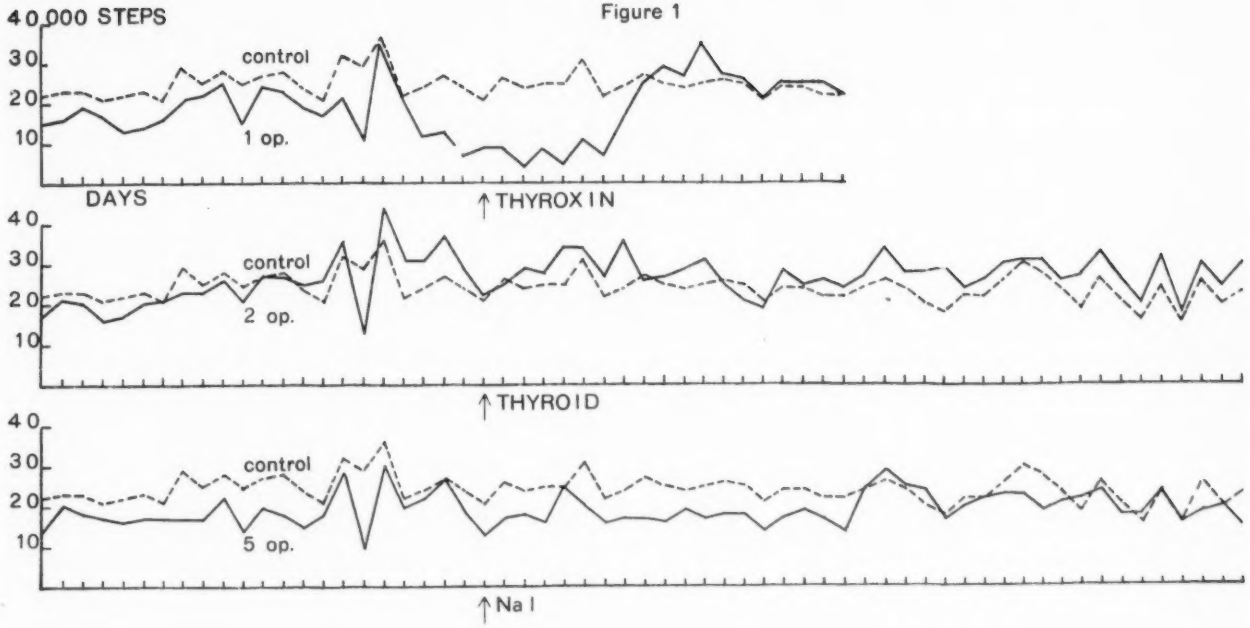
Activity records of thyroidectomized sheep following thyroxin administration

DAYS	ANIMAL					
	4op	4op	1op	9op	11op	5op
1 thyroxin begun	5,250	16,166	8,629	6,952	19,737	
2	3,750	19,041	8,629	3,689	19,383	10,409
3	7,500	12,814	4,315	5,391	19,501	9,623
4	7,250	13,412	9,152	4,114	19,028	12,623
5	8,875	*29,698	4,667	2,270	16,665	9,236
6	7,625	26,944	11,041	7,378	*34,865	10,597
7	3,750	22,274	7,155	1,419	28,011	*14,011
8		20,597	*15,656	*12,059	35,929	16,399
9	3,000	23,711	24,952		20,801	13,152
10	*12,375	26,226	29,355		29,529	14,388
11	12,125	24,908	27,276	13,053	24,318	11,456
12	14,500	24,676	35,348	15,606	31,266	14,901
13	15,250	24,546	27,398	11,634	26,923	15,079

* The asterisk indicates the first significant increase in activity following thyroxin.

Fig. 1. Graphs showing the effect of sodium iodide, thyroid extract, and thyroxin, respectively, on the spontaneous activity of three cretin sheep. The broken lines show the average daily activity of three normal animals while the solid lines indicate the activity of each of the three thyroidectomized sheep. The arrow in each graph notes the first administration of the test substance.

Figure 1


40,000 STEPS
30
20
10
control
1 op.
DAYS
↑THYROXIN
40
30
20
10
control
2 op.
↑THYROID
40
30
20
10
control
5 op.
↑NaI

decrease in the girth of the abdomen which can be most easily explained as a result of the increased tone of the striated muscles of the abdominal wall.

The latent period, described above, shows no definite relation either to the age of the animal or to the amount of thyroxin injected. (See table 1.) Its significance is unknown. The most probable explanation is that the thyroxin must undergo some chemical transformation within the body before it can take the place of the normal thyroid secretion.

Thyroid extract is known to be efficacious in thyroid deficiency and it is also generally believed that iodine is the essential element of the thyroid secretion. An experiment was therefore planned to compare the effects of thyroxin, thyroid extract and sodium iodide respectively, on the spontaneous activity of thyroidectomized sheep. A preliminary account of this experiment has been published (2). A flock of normal and cretin sheep were pastured in a field of about 20 acres. Once each day they were driven to a small pen where their pedometers were read. For twenty-two days the spontaneous activity of three pairs of twin sheep was recorded daily. One animal of each of these pairs had been thyroidectomized when about one month of age and all three cretin animals were distinctly dwarfed. On October 22, 1922, thyroid therapy was begun. To one of the cretin sheep, 1op, 0.25 mgm. of thyroxin was administered by subcutaneous injection every other day. To another, 2op, 0.3 gram of Parke Davis thyroid extract was fed in a gelatine capsule every other day and to a third, 5op, 0.5 gram of sodium iodide was also fed in a capsule every other day.

The effect of each of these substances on the spontaneous activity of the cretin sheep is shown in figure 1. The number of steps is recorded on the ordinate and the divisions on the abscissa indicate days. The broken lines mark the average activity of three normal sheep with which the activity of each of the three thyroidectomized sheep, indicated by the continuous lines, can be compared.

Cretin sheep 5op remained consistently below the average in daily activity and the feeding of sodium iodide clearly had no effect. Cretin sheep 2op although it tended to remain below the average in spontaneous activity, exhibited less depression than 5op. The feeding of thyroid extract produced no sudden effect on the neuro-muscular activity of this animal. However, a general increase in the number of steps taken from day to day is noticeable. The thyroidectomized sheep, 1op, during this

Fig. 2. Moving pictures of a cretin lamb 9 months old, thyroidectomized at 17 days of age. The two strips of film at the left (to be read from top to bottom) picture the lamb before the administration of thyroxin. Its muscular weakness is apparent. The two strips at the right show the same animal with its twin control after three weeks' treatment with thyroxin. The moving pictures were taken by Mr. B. R. Macmillan of the Department of Physiology.


Produced at
Physiological Field Station,
Cornell University
Medical College, Ithaca, N. Y.
Photographed by
Mr. B. R. Macmillan
of the physiology department.

experiment suffered one of its attacks of great muscular weakness and lethargy. When its activity was at the lowest level the subcutaneous injection of thyroxin was begun. The latent period already described is noticeable but after six days of continued feeble activity a sudden sharp rise in the curve is observed until within ten days of the beginning of the injections the cretin's activity is above the average. Unfortunately dogs gained entrance to the pasture and killed the animal. However, it is certain from previous experience with thyroxin that the increased activity would have been maintained even for a long period following the last injection.

The most interesting features of this experiment are first, the absence of any effect on spontaneous activity of the continued ingestion of sodium iodide. This substance certainly cannot take the place of the normal thyroid secretion. Nor can it, in the absence of the thyroid, be elaborated by any other tissue into the potent thyroid secretion. Second, thyroid extract exerts an effect on the neuro-muscular activity of an animal suffering thyroid deficiency but no latent period is observed as in the case of thyroxin. This may mean that substances in the extract are so similar to the normal thyroid hormone that little transformation is required within the body before the extract can produce an effect similar to the normal secretion.

SUMMARY

1. The subcutaneous injection of small amounts of thyroxin (0.1 to 0.5 mgm. daily) causes a sudden and large increase in the muscular power and neuro-muscular activity of thyroidectomized sheep even during extreme lethargy and muscular weakness.

2. This increase occurs only after a latent period of 3 to 8 days. The length of the latent period is not directly related either to the dose of thyroxin or to the age of the animal.

3. Although thyroid extract increases the neuro-muscular activity of the cretin sheep no latent period is observed.

4. Sodium iodide has no effect on the activity of the thyroidectomized sheep.

The expenses of this research were in part defrayed by a grant from the Heckscher Research Foundation of Cornell University.

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THE RELATION OF VITAMIN DEFICIENCY TO MUSCLE FATIGUE

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Received for publication December 26, 1924

In addition to the well-known constituents such as proteins, inorganic salts, fats and carbohydrates, a diet must contain certain chemically unknown factors that have received the name vitamins. Data have accumulated emphasizing the necessity of these substances for growth and general well-being for various classes of animals.

It was early recognized that an animal deprived of any of the vitamins would exhibit sooner or later certain typical symptoms which are specific for the characteristic vitamin deficient in the diet. Thus a deficiency of vitamin A in the diet, notwithstanding the fact that all the other components are optimum, results in a condition known as xerophthalmia, a peculiar eye affliction which eventually results in total blindness, unless the missing vitamin is added. An animal deprived of vitamin B develops a condition known as polyneuritis; the same disease which in man is called beri-beri. When vitamin C is either absent or present in too small quantity scurvy develops, although it is believed at the present time that this vitamin is only necessary in the diet of man, guinea pig and monkey. Recent work indicates that another vitamin known as vitamin D is necessary in addition to the above three for the normal functions of the body.

Although we may know comparatively little as regards the functions of these unknown constituents in metabolism, still we know something about the pathological conditions which result when they are absent. That animals on diets deficient in vitamin B develop polyneuritis, in which the coördinating powers of the muscles are lost, is known, and that this loss of control is the result of degeneration of some part of the nervous system has been suggested. To what extent or what parts of the nervous system are affected is still unknown.

McCarrison (1) believes that the nervous symptoms resulting in polyneuritis columbarum are the outcome not so much from a peripheral neuritis as from a disturbance in the function of cell groups within the sphere of influence of the cerebellum and resulting from malnutrition. According to McCarrison, the fact that vitamin extracts relieve the

symptoms of polyneuritis columbarum so quickly indicates that there are no extensive degenerative changes in the nervous system. This is substantiated by histological study. Cowgill (2) found on examination of the leg muscles of dogs suffering from paralytic symptoms as a result of vitamin B deficiency a condition of tonic spasticity, and he interpreted the loss of motion in the limb due not to lesions of the cells of the central nervous system, but rather to the presence of some toxic substance in the muscles. The tonic spasticity of the muscles disappears when the animal is anesthetized by ether; another fact that is in accord with the idea that a toxic substance is responsible for the symptoms. Walshe (3) maintains that the symptoms of beri-beri are the result of the production of a toxic substance which then affects the nervous system and other organs of the body. The origin and nature of the toxic substance are not known. McCarrison states: "There is no doubt that the starch component of the food hastens the onset of symptoms both of the pre-neuritic and the neuritic stage; that it does so by overloading the oxidative mechanism in the presence of endocrine insufficiency seems probable; but that it contributes a toxic factor which causes the nervous symptoms is in my opinion unproven." Uhlman (4) reports an investigation on rabbits and dogs where he administered in various ways commercial preparations of vitamin B and found among other results a marked improvement in muscle tonus, a lowering of blood pressure, and a stimulation of the cerebral and vagus nerves. Dutcher (5) is of the opinion that the function of vitamin B is that of a metabolic stimulant. He found that the body temperature fell during the course of polyneuritis and rose after the administration of the vitamin. He maintains that as a result of the depletion of the vitamin in the tissues the catalase content of the organs is considerably decreased. Findlay (6) states that glyoxalase in the liver is markedly reduced in polyneuritic pigeons. On the other hand Burge and Neill (7) state that the catalase content of the tissues varies considerably in the same species so that one is hardly justified in attributing too great an importance to the relation of vitamin B to oxidative powers in the animal body. The peculiar train of symptoms manifested by animals suffering from polyneuritis has led investigators to the idea that the lesions in this disease are situated principally in the nervous system, although McCarrison (1) points out that practically every tissue in the body suffers degeneration, and that certain disorders of the intestinal tract are more severe and perhaps of greater importance than the nerve lesions. However, the nature of the lesions in the nervous system is not known. No doubt future investigations concerning the pathology of the animal body deprived of the other vitamins as A, C and D will reveal the fact that although a specific effect is produced by the absence of these vitamins which seems to outshadow other disorders, nevertheless degeneration may be the result in practically all of

the tissues of the body. The object of the experiments reported in this paper was to determine, if possible, the effect of a deficiency of vitamins in the diet on muscle fatigue.

EXPERIMENTAL. All of the experiments recorded were performed on rats. Prior to being placed on the respective diets the animals had been on a good growing ration supplemented with whole milk. This ration has been used very successfully in this laboratory for breeding animals for the past five years. The deficient rations which were employed were of such a character as to be low or lacking completely either in vitamins A or B but otherwise these rations were satisfactory. The vitamin B deficient ration had the following composition: alcoholic extracted casein 18 per cent, filtered butterfat 5 per cent, salt mixture 185 (8), 3.7 per cent, and dextrin 73.3 per cent. This ration is designated in the text as number 3. The vitamin A deficient diet consisted of alcoholic extracted casein 18 per cent, wheat embryo 5 per cent, salt mixture 185, 3.7 per cent, and dextrin to 100 per cent. This ration is labeled number 4 in the records. It is necessary to free the casein completely from vitamins A and B, since commercial casein contains varying quantities of these two vitamins which would otherwise influence the results. The casein was therefore extracted with 95 per cent ethyl alcohol, preferably in large percolators equipped with a siphon arrangement similar to a Soxhlet extractor. No casein was employed in these deficient rations, the vitamins of which had not been removed. The dextrin was prepared by moistening ordinary starch with one per cent solution of citric acid and autoclaving at twenty pounds pressure for three hours. The results obtained with these diets were compared with rations which were complete as far as the nutrition of the rat is concerned judged by the ability of the weaned animal to grow to maturity at the normal rate. Recent work by Nelson, Heller and Fulmer (9) show that diets may contain all of the components necessary for growth and general well being and still not be optimum for normal functioning. Such diets reveal their inadequacy during the suckling period. However, this fact need not enter into consideration here since these experiments are mainly concerned with effects produced by the presence or absence of vitamins A and B. Complete diet number 1 consisted of commercial casein 20 per cent, filtered butterfat 5 per cent, wheat embryo 5 per cent, salt mixture 185, 3.7 per cent, and dextrin 66.3 per cent. Complete purified ration number 2 consisted of alcoholic extracted casein 18 per cent, filtered butterfat 5 per cent, wheat embryo 5 per cent, salt mixture 185, 3.7 per cent, and dextrin to 100 per cent. Complete ration number 7 was the usual one used in this laboratory for all breeding stock.

Before taking the kymograph records of fatigue on individuals, observations were made and records kept of temperature, heart rate and respiration. A few rats were put to death by the administration of chloro-

form, but the majority were killed by a quickly executed blow on the head, since it was found that chloroform markedly affected the form of the fatigue curve. The rat muscle fatigue curves produced by the chloroform method of killing were similar in contour to those obtained by Baldwin (10) on frog muscle when the latter is immersed in strong ethyl alcohol. Because of its innervation and the facility with which it can be handled experimentally, the gastrocnemius muscle was used. The skin of the leg was quickly removed, and while the muscles were being bathed in warm Locke's solution, the sciatic nerve was isolated. The tendon of Achilles was severed from the foot and the distal portion of the leg was severed and removed from the knee. The nerve was carefully lifted from the underlying adductor magnus muscle by a warm glass hook and the whole preparation finally mounted in such a way as to obtain tracings upon the drum. A special technique was developed in indirect stimulation which consisted of mounting a glass rod in such a position as to lift the nerve out where the platinum electrodes could be applied with assurance of proper contact and to obviate any possibility of contact with the underlying muscles. Direct and indirect methods of stimulation were used and in all cases technical precautions were observed to keep the preparation moist and warm. The rate and strength of stimulation were uniform throughout and were regulated by a mounted chronometer clicking seconds recording upon the drum. A chronometer clicking minutes and recording, served as a time marker. The drum speed was calibrated and its rotation uniform for all the experiments.

Although a large number of fatigue records were obtained by the kymographic method, only the most typical need to be analyzed. By varying the technique it was often possible to obtain two records from an individual. Frequently the stimulation was applied indirectly through the nerve of one leg and directly through the muscle on the other; in other cases both were stimulated directly or indirectly as desired. The effect of indirect stimulation through the nerve will be reported upon subsequently. This paper is concerned with direct stimulation only. Because of the close correspondence of resulting curves on the two legs treated similarly, it was found a waste of time to make a practice of this procedure, except in special cases where checks were desirable. Preliminary observations showed clearly that no marked differences resulted in curves when the muscle was directly stimulated in the air from those which were totally immersed in Locke's solution provided the former were kept moist during the interval of fatigue which varied from a few seconds to not more than six minutes.

In analyzing fatigue curves it is convenient to begin with so-called normal rats on growing rations. These records closely resemble frog muscle curves in amplitude and duration, and also in their various contour phases. There is usually a rapid initial contracture, followed by a period of gradual

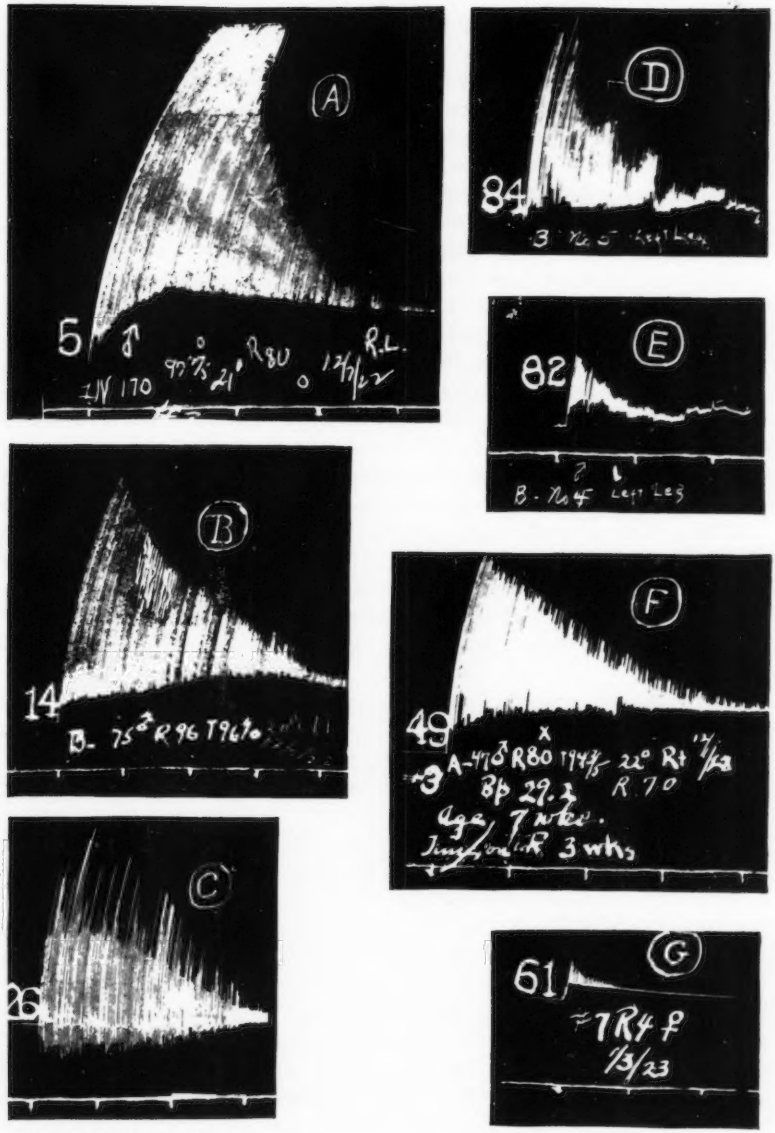

Fig. 1. Typical fatigue curves.

A. Normal male rat weighing 170 grams on diet containing all essential food factors.

B. Rat on a vitamin-B-deficient diet for three weeks. Weight 75 grams.

C. Male rat on a vitamin-B-deficient diet for six weeks.

D. Male rat on a vitamin-B-deficient diet for eight weeks.

E. Rat on a vitamin-B-deficient diet for ten weeks.

F. Rat on a vitamin-A-deficient diet for three weeks.

G. Rat on a vitamin-A-deficient diet for six weeks.

shortening during which time the most extensive strokes of the lever are made. A typical curve illustrating these points on direct stimulation is shown in tracing *A* of figure 1. This curve was obtained from a male rat weighing 170 grams, on the complete synthetic diet no. 1. The duration of the fatigue period in this instance was about four minutes. On a growing ration supplemented with milk, some differences are usually noted, the most marked being the prolongation of the efficiency span.

The experiments on animals on diets deficient in vitamin B were made at different intervals of time after being placed upon the rations. The first series were performed on animals on the deficient diet for three weeks, and a typical direct stimulation fatigue curve is shown in tracing *B*. This particular male animal weighed 75 grams, and the curve of growth was decidedly characteristic for this type of diet. The rat's temperature at the time was 96.5°F. which is subnormal for rats of equal age. On analysis the performance of the muscle is fairly good. The curve shows that the muscle had good tonicity, a duration of about three minutes, and a slight tendency toward contracture toward the end. It lacks, however, the efficiency span during the initial contractions which is so characteristic of the curves of normal and milk fed animals as just discussed. There is a slight indication of irregularity in contracture as evidenced in the upper contour of the curve and this is more and more pronounced in animals on diets for longer periods of time as will be seen subsequently. On a comparative basis one is tempted to interpret this to mean that, although the results are not extreme, the lack of the vitamin for this interval of time begins to show its physiological effect. Tracing *C*, which is a typical record of a male rat, weighing 65 grams, shows a decided loss of tonicity and possesses erratic responses which are very characteristic of animals on vitamin B deficient diets for six weeks. The amplitude, as well as the duration, is greatly reduced. The lack of tonicity is marked and the contour is very irregular. At the time of the experiment the subject's temperature was 96.8°F. A decided break in the growth curve occurred at the end of the third week on the diet. Typical records were obtained from rats on vitamin B deficient rations for eight weeks. Tracing *D* shows a curve the form of which is very irregular. This was a male rat twelve weeks old, weight 97 grams, having a muscle response of only three minutes' duration. The subject's temperature was 97.6°F. Animals on vitamin B deficient diets ten weeks or more are as a rule so weak that only seldom do they care to move about. Tracing *E* is a curve selected from a considerable number on this type of diet for this length of time. This animal was typical of the entire group and from the fatigue curve shown in tracing *E* it is noticed that its response was very feeble, in spite of the fact that the muscle in this case was weighted only with a rubber band. The duration interval was but two minutes; the subject's temperature was 96.8°F.

From a group of rats that were put on a diet deficient in vitamin A for three weeks we have selected a male rat weighing 47 grams. It had a temperature of 94.4°F. The fatigue record of this rat is shown in tracing *F*. There is, in most cases, as typified in this record, a tendency to spasmodic contracture especially toward the close of the fatigue process, but it is not nearly as conspicuous as in the records of animals suffering from vitamin B deficiency. Tonicity seems in these animals to be fairly well maintained, but apparently there is little reserve so that the efficiency interval is lacking and the onset of fatigue is rapid and progressive throughout. Animals maintained on vitamin A deficient diets for six weeks show marked pathological symptoms, as a rule, and from the fatigue standpoint are scarcely able to perform at all. A typical curve is shown in tracing *G*. This curve was obtained from a female rat weighing 85 grams, having a subnormal temperature of 96.1°F. It shows the initial contracture so characteristic of all vitamin A deficient records, but in amplitude is so reduced that only a few strokes are effective. The fatigue is completed in less than a minute. The animals in this group showed typical symptoms of xerophthalmia.

SUMMARY

1. Fatigue curves produced by animals on diets lacking vitamin B show progressive diminution in amplitude and endurance as the length of time on the diet increases. These curves also show progressive loss of tonicity and an irregularity of contracture that seem very characteristic.

2. Animals lacking vitamin A produce similar curves which in amplitude and duration are roughly inversely proportional to the interval of time on the diet, but the decrease in tonicity is not so apparent.

3. Just what mechanisms are affected by vitamin deficiency still remains to be learned, but it would seem that upon direct stimulation the muscles are certainly involved.

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THE DISTRIBUTION OF GLUCOSE BETWEEN HUMAN BLOOD PLASMA AND RED CORPUSCLES AND THE RAPIDITY OF ITS PENETRATION

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Received for publication December 13, 1924

Among the many questions concerning the occurrence of glucose in the blood, that of its distribution between the plasma and the formed elements is one of the most debated.

In a contribution by one of us (1) will be found a discussion of the more important contributions to the earlier literature. The result of this investigation was, as far as the question of the distribution of blood sugar is concerned, a confirmation and continuation of Kozawa's (2) and of Masing's (3) results, so that one may now feel justified in adopting the following conclusions:

1. The corpuscle membranes of all animals thus far investigated are impermeable to glucose.

2. Human corpuscle membranes are permeable to glucose.

3. The distribution of glucose between human corpuscles and plasma, for normal concentrations of glucose, is about 75:100, a relation which finds possible explanation in the distribution of water between corpuscles and plasma, which is also about 75:100.

4. Because of certain discrepancies between the corpuscle-sugar concentrations obtained by direct analysis and those obtained by calculation from osmotic relationships (corpuscle volume changes, hemolysis, etc.) it may be assumed, according to Ege, that glucose not only penetrates into the corpuscles but is also adsorbed upon the outer surfaces of the corpuscle membrane.

5. The true rapidity of penetration seems to be very small.

The truth of these conclusions has been both confirmed and discredited by later investigations. Thus it has been insisted that human corpuscles, under normal physiological conditions are impermeable to glucose, that the glucose concentration in the corpuscles of the circulating blood is zero (4), (5), (6), and that during coagulation or by the use of anti-coagulating agents the membrane is so altered as to become permeable. The correctness of this view, however, is not indisputable, for another series

of investigations has avoided those anti-coagulating agents (Ca-precipitating salts) which, according to Falta and Richter-Quittner, are the cause of the alteration of the membrane (7), (8). Using the same technique as did Brinkmann and van Dam (collection of the blood in paraffined tubes), whereby the blood is obtained in as near the natural condition as possible, there has been found (9), (10) a considerable concentration of sugar in the corpuscles, a result which we have also been able to confirm. Finally, Bürger has determined the distribution of glucose in hemophilic patients and found approximately equal concentrations in the corpuscles and plasma of the absolutely undisturbed blood. It would seem, therefore, that there is no longer reason to believe in the sugar-free human corpuscle. (See also Hendrix and Sweet (11) for the case of lymph.)

There still remains, however, the question of the exact relation between the glucose concentrations in plasma and corpuscles, for here also there is some disagreement. Masing, Ege and Hagedorn all find a relatively constant distribution relation of corpuscle-sugar to plasma-sugar (C/P) of from 70:100 to 80:100. Others have found a little more variable ratio in the neighborhood of 100:100. Fitz and Bock (12), Wu (13) and Folin and Berglund (14) find on the average a little under 100:100; Rakestraw (15), a trifle over.

The question now is whether these divergences represent anything more than accidental analytical errors. With a corpuscle volume of 40 per cent an error of 5 per cent in the whole blood and plasma sugar will, in the most extreme case, cause uncertainty of the distribution ratio between 75:100 and 100:100. From de Wesselow's work (16) there is another possible explanation for these discrepancies. According to him there are other substances in the corpuscles (creatinine?) which have considerable reducing power if one used Benedict's picric acid method but very little reducing action in Maclean's method. By Benedict's method C/P may be found to be greater than 100:100 while by Maclean's method on the same blood C/P may be less. The "residual reduction" of the Hagedorn-Jensen method used in our work has been shown to be of small importance for normal blood; i.e., 3 to 5 per cent (17).

Tachau (18) is of the opinion that the various distribution coefficients which are found may be explained on the assumption that the equalization of the glucose concentration between plasma and corpuscles takes appreciable time. If the sugar concentration in the blood is increasing one will find the greater portion in the plasma; if it is falling there will be opportunity for a greater concentration in the corpuscles. Tachau's reasoning seems to find confirmation in the investigations of Masing, of Kozawa, and of Ege on the rapidity with which glucose penetrates human corpuscles in vitro. His theory also seems to be supported for normal individuals by the thorough-going work of Karen Marie Hansen (10).

But now we come to a problem which makes it necessary to know the glucose relation at equilibrium and the rapidity with which equilibrium is reached.

Karen Hansen finds that the distribution in normal fasting individuals is 100:100, while for fasting diabetics it is about 80:100, and further,

TABLE 1

	SUBJECT		GLUCOSE IN PLASMA	GLUCOSE IN WHOLE BLOOD	CORPUSCLE VOLUME	GLUCOSE IN CORPUSCLES	C/P
			per cent	*per cent*	*per cent*	*per cent*	
1	P.*	Collected in paraffined tube	0.107	0.095	46	0.081	**76**
		Collected in 0.1 per cent oxalate	0.101	0.096		0.090	89
2	E.*	Oxalate. Analyzed immediately	0.096	0.089	45	0.080	**83**
		Same after standing 8 hours at room temperature	0.084	0.082		0.080	**95**
3	H.	In paraffined tube. Analyzed immediately	0.112	0.099	45	0.083	**74**
		Oxalate. After 5 hours at 40°	0.091	0.081		0.069	76
4	Mo.	In paraffined tube	0.099	0.089	41	0.074	**75**
		Oxalate. After 2½ hours at room temperature	0.089	0.083		0.074	83
5	E.*	In paraffined tube. Analyzed immediately	0.105	0.095	46	0.084	**80**
6	G.	In paraffined tube. Analyzed immediately	0.090	0.081	39	0.064	**74**
7	E.	In oxalate. Analyzed immediately	0.095	0.085	50	0.075	**79**
		Oxalate. After 2 hours at room temperature	0.085	0.076		0.065	77
8	R.	In oxalate. Analyzed immediately	0.071	0.065		0.058	**81**
9	Me.	In oxalate. Analyzed immediately	0.096	0.091	54	0.087	**91**

* Fasting.

that the relation in normals varies after ingestion of glucose. As concerns the lower ratio in diabetics one may also mention the work of H. J. John (19) whose analytical results, however, seem to be rather uncertain.

Glucose distribution in humans. From earlier experiments it seems to follow that the distribution coefficient is not appreciably different whether one uses fluoride, oxalate or hirudin as anti-coagulant. Furthermore, no systematic difference in the coefficient is found whether the blood is collected in oxalate or in paraffined tubes with rapid centrifugation, so that the plasma and corpuscles are separated and ready for analysis within five minutes. It is also known that allowing the blood to stand in oxalate after collecting has no influence upon the distribution coefficient, from which it may be concluded (see also below) that the first analysis corresponds to a condition of equilibrium.

Table 1 shows that the initial distribution coefficient in normal individuals lies between 74 and 95 (average 79). Variations within this range are of little significance. It may also be seen that it makes no difference whether the blood is collected in oxalate or in paraffined tubes (nos. 2 and 5), in fasting condition or a few hours after the last meal (nos. 2 and 7). Standing is also seen to produce no change, and stasis is similarly without effect.

We are therefore unable to explain how Karen Hansen, using the same technical and analytical methods, found that a distribution coefficient of a certain magnitude was characteristic of diabetics or of individuals who were related to diabetics. All of our subjects were normal (certainly not diabetic), and in only one case was there any diabetes in the immediate family.

Rapidity of penetration. It will also be of interest to determine the rapidity with which sugar, added to the outer plasma, distributes itself between this and the corpuscles and to see at what concentration equilibrium results. Experiments are made in the following manner:

One cubic centimeter of oxalated blood is mixed with about 20 cc. of 5.5 per cent glucose solution (isosmotic concentration) and maintained at a constant temperature in a water bath, the corpuscles being held in suspension by passing a slow stream of air through the solution. Samples of 1 to 2 cc. are removed at occasional intervals, cooled and centrifuged in a Hamburger conohematocrit (20) until the corpuscle column in the graduated capillary is entirely transparent, which is an indication that the corpuscles are quantitatively separated. The supernatant solution is sucked off and the tube rinsed with 0.9 per cent NaCl solution to remove the last traces of glucose. This can be done without danger of withdrawing glucose from the corpuscles, for the upper surface of the corpuscle column is very small. The procedure is carried out at room temperature and takes very little time. A little distilled water is added, the corpuscle column carefully and quantitatively sucked out and used for the sugar analysis. The sugar concentration in the plasma-medium may likewise be determined, although this will not change appreciably

during the experiment. Since the capillary tube is accurately calibrated one knows the absolute quantity of corpuscles used for analysis.

In addition, the changes in volume of the corpuscles which take place when suspended in a glucose solution of definite concentration may also be followed. This was the object of our investigation, since earlier experiments (21) had shown a discrepancy between sugar concentrations determined in the corpuscles by direct analysis and those calculated from the osmotic pressure of the sugar solution, excluding the possibility of loss of salts or adsorption of glucose. (This calculation assumes a water phase of about 80 per cent.)

Experiment at 41°C. Blood from E.

One cubic centimeter of blood (oxalated) + 20 cc. of 5.5 per cent glucose + 5 cc. Sörensen's phosphate mixture, pH = 6.8.

Samples taken 18 minutes after mixing showed a considerable hemolysis. From this it may be concluded that the glucose penetrates so rapidly into the corpuscles under these conditions that the tension within is more than half as great as the tension in the plasma-medium. In order to follow the penetration of the glucose one must therefore work at a lower temperature.

Experiment with same blood at 30.3°C.

One cubic centimeter of blood + 25 cc. of 5.5 per cent glucose. One cubic centimeter samples of the mixture centrifuged and analyzed.

MINUTES AFTER MIXING	VOLUME OF CORPUSCLES USED	DITTO, IN PER CENT OF ITS VOLUME IN ISOTONIC NaCl SOLUTION	GLUCOSE FOUND	
	cu. mm.		*mgm.*	*per cent*
2	19.2	99	0.238	1.24
18	21.2	112	0.576	2.64
49	23.6	122	0.798	3.38
72	25.5	131	0.912	3.54
154	28.7	148		
224	29.1*	153	1.24	4.4

* 27.5 cu. mm. used for analysis; beginning hemolysis.

Since the corpuscles are suspended in a pure glucose solution isosmotic with the plasma they will swell gradually as the glucose penetrates into them until hemolysis finally results. In a 0.45 per cent NaCl solution the corpuscles swell to about 150, their volume in a 0.9 per cent NaCl solution being taken as 100.[1] Further swelling will generally produce

[1] Calculated from the equation: $P\ (100 - x) = P_1\ (V_1 - x)$, where P and P_1 are the osmotic concentrations of 0.9 per cent and 0.45 per cent NaCl solutions, respectively; V_1 is the unknown volume in 0.45 per cent NaCl, and x the disperse phase in the corpuscles, 40 to 50 per cent. See (21).

hemolysis. When the corpuscles have swollen to 153, as in the last determination in the above experiment, the glucose tension within the corpuscles must be only half as great as that in the outer plasma-medium. Direct analysis, however, shows the relation between glucose in the corpuscles and in the plasma-medium to be 4.4:5.5 = 80. When one takes into consideration the disperse phase of the corpuscles this must obviously indicate that approximate equilibrium has been reached between the glucose tensions within and without, and that the corpuscles should already have been hemolyzed.

In an ealier work one of us has shown that this discrepancy may be explained on the assumption that the glucose analyses do not give a correct measure of the glucose tensions within the corpuscles, since it is possible that part of the glucose associated with the corpuscles may be adsorbed on the membrane surfaces. There is also another possible explanation; namely, that coincident with the penetration of glucose there may also take place an effusion of salts from the corpuscles. That such an effusion does take place when the corpuscles are suspended in a solution of an anelectrolyte has been shown by Calugareanu and Henri, among others (22). But whether this effusion is considerable enough to explain the discrepancy mentioned above has not been investigated.

From the experiments we see that at 30° glucose penetrates the corpuscle membranes rather slowly, since equilibrium is reached only after three to four hours. At 40° the penetration is very much more rapid; equilibrium is reached in about twenty minutes. The rapidity of penetration thus increases more han ten-fold for 10° rise in temperature. At lower temperatures penetration is of course still slower.

Experiment at 20°. Oxalated blood from R.
One cubic centimeter in 25 cc. of 5.5 per cent glucose.

MINUTES AFTER MIXING	CORPUSCLE VOLUME IN PER CENT OF THE VOLUME IN 9 PER CENT NaCl SOLUTION	GLUCOSE CONCENTRATION BY ANALYSIS
6	89.3	0.80
34	96.5	1.65
58	100.0	2.19
88	100.0	2.58
126	106.0	2.98
230	124.0	3.10

No direct relation can be drawn between this experiment and the foregoing ones, since the blood has been taken from different individuals, and from a series of previous experiments there is some reason to believe that the rapidity of penetration varies with different individuals.

Of still greater interest is the influence of the surrounding medium upon the rapidity of penetration of glucose.

Experiment at 30°. Oxalated blood from E.

15 cc. + 0.5 cc. of 6 per cent glucose. Samples removed after one minute, centrifuged for a few moments, plasma withdrawn and the usual analyses made upon plasma and whole blood.

MINUTES AFTER MIXING	PER CENT GLUCOSE IN			C/P
	Plasma	Whole Blood	Corpuscles*	
1	0.306	0.271	0.240	78
20	0.301	0.272	0.245	81
185	0.273	0.251	0.230	84

* Corpuscle volume = 53 per cent.

The variations found in C/P are small enough to be within the limits of error.

While the earlier experiment with the blood from E shows that from three to four hours elapse before the glucose has penetrated to a condition of equilibrium (C/P = 80) at 30°C. when the surrounding medium is a pure solution of an anelectrolyte, here we see that equilibrium results at the same temperature and with the same blood in less than one minute when the surrounding medium is oxalated plasma. From this it may be concluded that the distribution of glucose between plasma and corpuscles in vivo at 37° must take place so rapidly that equilibrium is reached before samples can be removed for analysis. This assumes, of course, that the precipitation of Ca has no influence upon the penetration of glucose. A further investigation is needed, however, to determine the factors causing this great difference between the penetration from pure solutions and from oxalated plasma.

SUMMARY

1. The distribution of glucose between red blood corpuscles and plasma of normal humans is about 80:100.

2. The distribution is the same whether coagulation is prevented by addition of oxalate or by collection in paraffined tubes. Standing, and the resulting glucolysis, have no important influence upon the distribution ratio.

3. Added glucose, at the blood's temperature, is distributed almost instantaneously between plasma and corpuscles.

4. When corpuscles are suspended in pure glucose solution the penetration of glucose is much slower.

5. Temperature has an extremely great influence upon the rapidity of penetration.

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THE RELATION BETWEEN THE STIMULATING EFFICIENCY OF INTERMITTENT AND CONTINUOUS LIGHT

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Received for publication December 29, 1924

It has been demonstrated in the butterfly, Vanessa antiopa, the tachina-fly, Archytas aterrima and the drone-fly, Eristalis tenax, that under certain conditions the stimulating efficiency of intermittent light is considerably higher than that of continuous light. In the following pages we shall deal quantitatively with the magnitude of this difference. The results obtained have an important bearing on the explanation of the processes involved in stimulation.

The methods of experimentation were in general like those described in an earlier paper. The essential features of the apparatus used consisted of two 1000 watt, 110 volt, monoplane-filament, stereopticon lamps so mounted and screened as to produce two horizontal beams of light which crossed at right angles in the field of observation and two rotating sector-disks, one in each beam (4, fig. 1).

The observations were made as follows: The openings in each of the two sector-disks were adjusted so as to produce a ratio between the length of the light and the dark periods of 1/15; the two lamps were arranged so that each, with the disk rotating, produced at the center of the field of observation an illumination of 115 m.c., and the speed of both motors connected with the sector-disks was regulated to produce 125 flashes per second in each beam. Now, from a group of specimens of Eristalis, which had been collected a few hours earlier in the morning and dark adapted, ten which oriented fairly accurately were selected and tested successively in the field of light, each making six paths as described in the preceding paper (5).

Thus there were obtained records of six paths for each of the ten specimens showing the deflection from the diagonal between the two beams of light both of which had a stimulating efficiency known to be equal to that of continuous illumination. The flash-frequency of the light in one of the beams was now reduced to 20 per second, that of the light in the other, hereafter designated continuous, remaining at 125 per second. Then

the ten specimens, now completely dark adapted, were again tested precisely as before and in the same order.

The opening in the sector-disk in the beam of continuous light was now increased so as to increase the illumination, all other conditions remaining the same, after which the ten specimens were again tested as before. This was repeated with various luminous intensities until there were obtained records of a group of six paths for each of the following illuminations in the beam of continuous light: 115, 230, 460, 920, 1840 and 3680 m.c., the light in the other beam remaining at 115 m.c. with a flash-frequency of 20 per second, and a ratio between the length of the light and the dark periods of 1/15. Luminous intensity of 3680 m.c. in the beam of continuous light was obtained by decreasing the distance between the lamp and the field of observation, all of the other intensities by changing the size of the opening in the sector-disk.

TABLE 1

Relation between stimulating efficiency of continuous and intermittent light in luminous intensity of 115 m.c.

i, deflection toward intermittent light; *c*, toward continuous light

FLASH FREQUENCY PER SECOND INTERMITTENT LIGHT	RELATION IN ENERGY PER UNIT TIME		ANGLE OF DEFLECTION WITH LIGHT AND DARK PERIODS		
	Continuous light	Intermittent light	1 to 15	1 to 3	1 to 3
125	1	1	1.11 *i*	3.09 *i*	1.30 *c*
20	1	1	19.91 *i*	21.98 *i*	25.63 *i*
20	2	1	17.84 *i*	18.66 *i*	23.49 *i*
20	4	1	16.13 *i*	18.14 *i*	18.15 *i*
20	8	1	7.51 *i*	8.83 *i*	11.25 *i*
20	16	1	1.86 *i*	3.10 *i*	6.69 *i*
20	22	1	Not tested	0.48 *c*	Not tested
20	32	1	18.09 *c*	Not tested	Not tested

Similar groups of records were obtained for ten other freshly collected specimens under precisely the same conditions except that the ratio between the length of the light and the dark periods was 1/3 in place of 1/15, that the highest illumination was 2530 m.c., and that all illuminations above 460 m.c. were obtained by moving the lamp. Under these conditions, except that the two lamps were interchanged, similar groups of records were also obtained for ten other freshly collected specimens.

The total averages of these groups of records are presented in table 1, each number representing the angle of deflection under a given condition being the average of the deflection in 6 trials for each of 10 individuals, a total of 60 trials. This table shows that three series of averages were obtained, one with the ratio between the length of the light and the dark periods 1/15 and two with this ratio 1/3, and that these three series are

essentially the same. In the last series the two lamps were interchanged as previously stated. The fact that this series is in fairly close agreement with the preceding proves that the results presented were not due to possible differences in the two lamps.

The table also shows that with the flash-frequency of the intermittent light 125 per second, the illumination in the two beams being the same, the average angle of deflection was practically zero but that with the flash-frequency 20 per second there was a strong deflection toward the intermittent light. It shows, moreover, that as the intensity of the continuous light increased in relation to that of the intermittent light the average angle of deflection in all of the series of tests decreased to zero, after which it increased in the opposite direction, and that zero deflection was obtained when the illumination of the continuous light was between 16 and 22 times as intense as that of the intermittent light. This plainly indicates that at a flash-frequency of 20 per second in an illumination of approximately 115 m.c., the stimulating efficiency of intermittent light with the ratio between the length of the light and the dark periods 1/15 or 1/3, is more than 16 times as great as that of continuous light. In other words, that a given amount of light delivered intermittently at the rate of 20 flashes per second, with the dark periods 3 or 15 times as long as the light periods, has more than 16 times the stimulating effect of the same amount of light delivered continuously. What does this surprising result signify?

If there are in the nervous system or in the photoreceptors alternate sensitive and refractory periods in accord with the hypothesis presented in preceding papers (3), if in intermittent light under optimum stimulating conditions the sensitive and refractory periods coincide with the light and the dark periods respectively, if the stimulating efficiency is independent of the rate of reception of light, i.e., the intensity during the light period, and if the refractory period is constant under all conditions of illumination then the maximum stimulating efficiency of the intermittent light as used in the preceding experiments, i.e., 115 m.c. with a ratio between the length of the light and the dark periods of 1/3, should be only 7/4 times the stimulating efficiency of continuous light. Figure 1 makes this conclusion evident.

In this figure, $a'c'$ represents continuous light of 115 m.c. and ac intermittent light of the same total intensity with the light period, ab, 1/3 as long as the dark period, bc. If in this intermittent light the sensitive and refractory periods coincide with the light and the dark periods respectively, in accord with the assumptions made, it is evident that the sensitive period must equal ab, and the refractory period, bc; and if the stimulating efficiency is independent of the rate of reception of light then the sensitive period in continuous light must equal $a'b'$, for in intermittent

light the same amount of energy is delivered in *ab* as is delivered in continuous light in *a'b'*. If this is true, the sensitive period in the continuous light is four times as long as it is in the intermittent light. But if the refractory period is, in accord with the assumption made, independent of illumination, *bc* equals *b'c'*. If this is true, then, since *bc* equals 3/4 *a'b'*, the refractory period in continuous light, *b'c'*, is 3/4 as long as the sensitive period, *a'b'*, or 3/7 as long as the sum of the two periods. Therefore, if light has no effect during the refractory period, it is evident that 3/7 of the light received has no effect. Consequently, if all of the light received in intermittent light is effective the stimulating efficiency should be 7/4 times that of continuous light. We have found, however, as previously stated, that it is more than 16 times as great.

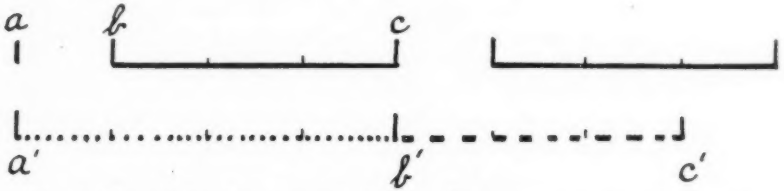

Fig. 1. The relation between stimulating efficiency in continuous and intermittent light expected under conditions stated in text.

If our hypothesis concerning the difference between the stimulating efficiency of intermittent and continuous light is valid this seems to indicate either that the refractory period varies inversely with illumination, being longer in continuous illumination where it occurs in light than in intermittent illumination where it occurs in darkness indicating that light retards restitution or that the effect of a given amount of light-energy received during the sensitive period varies directly with the intensity or both. In a preceding paper we have presented evidence which leads to the same conclusion.

SUMMARY

1. The stimulating efficiency of intermittent light of 115 m.c. with a flash-frequency of 20 per second and the length of the light and the dark periods 1 to 15 or 1 to 3 is more than 16 times as great as that of continuous light.

2. If the explanation presented in previous papers is valid this indicates that the effect of a given amount of light-energy received during the sensitive period varies directly with the intensity or that the refractory period is longer in light than in darkness or both.

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RHYTHMIC CONTRACTIONS IN ISOLATED STRIPS OF MAMMALIAN VENTRICLE

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Received for publication December 27, 1924

The problem considered in this investigation began with an anatomical study of the musculature of the mammalian heart.[1] In the course of our research the functional aspect of the subject became important. This report gives the results obtained by immersing isolated strips of the ventricular muscle in oxygenated solutions with the production of rhythmic activity of a pronounced character.

It is, indeed, well known that ventricular strips from the hearts of turtles and other cold-blooded animals may be immersed in solution and made to beat rhythmically. Furthermore, the isolated mammalian heart has long been studied—the entire heart has been perfused through the coronary vessels and thus made to beat; also, strips of the ventricle are known to beat rhythmically when perfused through the coronary vessels. But so far as the authors have been able to determine, isolated strips of mammalian ventricular muscle have never been found to give spontaneous rhythmic contractions after simple immersion in an oxygenated isotonic solution.

Our method is closely similar to that long used in the study of ventricular strips from cold-blooded animals. Thus, in 1880 Gaskell (7) proved that the lower two-thirds of the ventricle of a frog's heart would beat rhythmically when stimulated by a constant electric current, or when supplied with inorganic salt solution. Twenty years later Porter (10) demonstrated that strips of mammalian ventricle would give spontaneous rhythmic contractions when perfused through the coronaries. For the perfusion fluid he used an oxygenated solution of physiological saline and blood. Shortly after, Pratt (11), (12) demonstrated that the right ventricle of a cat heart would beat for some time if distended with blood, and suggested that the vessels of Thebesius might be an important source of nutrition in case of the obstruction of the terminal portions of the coronary vessels. Recently Crainicianu (4) has shown that when fresh human hearts are perfused with saline, a very considerable fraction of the perfusate entering the coronary arteries is expelled into the ventricles. This again

[1] The anatomical findings will be given in a subsequent anatomical report.

indicates that the connections of the coronary vessels with the interior of the ventricles must be more numerous than generally supposed.

Another decided step in the study of the mammalian heart was made by Erlanger (5) who took auricular strips from the cat's heart, and immersed them in pure Locke's solution. By this method, in response to electrical stimulation, he evoked rhythmic contractions which continued for several minutes after the removal of the stimulus.

We have excised small strips of ventricular muscle, and immersed them in a solution. By so doing we have succeeded in eliciting strong, rhythmic contractions for a period of several hours. Proper precautions, as detailed later, were taken as to solutions, temperature and tension.

By this method we have obtained rhythmic contractions from the following portions of the ventricles:

Left ventricle	*Animals used*
1. Anterior papillary muscle	Cat, kitten, dog, rabbit and man
2. Posterior papillary muscle	Cat and rabbit
3. Innermost muscular layer	Dog and sheep
4. Septal wall	Cat and sheep
5. Section through whole ventricle	Cat (adult and embryonic)
6. Circular muscle	Cat, dog and man
7. Apex	Cat
8. Outer layer (external layer)	Cat and dog
9. Purkinje tissue (Bundle of His)[2]	Calf and dog
Right ventricle	*Animals used*
1. Moderator band	Sheep
2. Section through right ventricle	Cat (adult, young, and embryonic)
3. Papillary muscle of right ventricle	Cat and dog

In short, we believe that any part of a mammalian ventricle can be studied by this method. The limitations are, presumably, the thickness of the strip and the viscosity of the immersion fluid.

This conclusion is based upon forty-six experiments. In most instances two strips were taken, so that nearly twice as many strips have been studied as are indicated by the number of experiments. Five of the forty-six experiments were failures—two on sheep hearts, one on human heart, two on cat hearts (one of which was on Purkinje tissue). Eight others were unsatisfactory, i.e., although the muscle strips beat, their behavior was irregular. The remaining thirty-one experiments were successful; in all of these muscle strips there were lasting rhythmic contractions.

In most experiments metallic instruments were used, and furthermore, the strips were suspended by metallic clips which had been previously coated with paraffin. At the suggestion of Dr. W. T. Porter we carried out a series of experiments in which no part of the heart or blood vessels was

[2] To be discussed in detail later.

touched by any metallic instrument to avoid the possibility of setting up an artificial electric current. Glass instruments were used throughout. The strips were attached to glass hooks and immersed in Ringer's solution. Linen threads were used between the hooks and writing lever. With this technique we obtained good rhythmic contractions and results entirely comparable to those in which metallic instruments were used.

The technique is very similar to that used by Erlanger in his study of mammalian auricular strips with the exception that the heart was never perfused and washed entirely free from blood preliminary to taking the muscle strips. On the contrary, it was found that 5 to 10 per cent of defibrinated blood added to the solution greatly increased the ease of eliciting spontaneous rhythmic contractions. Nevertheless, a high proportion of blood has not been found advantageous.

In addition to this two factors are important. The first is a low temperature (30° to 33°C.); the second is a light writing lever adjusted so that the two arms nearly balance. The first of these factors, a low temperature, Erlanger gives in his technique for studying auricular strips. Nevertheless, the importance of this temperature at the beginning of an experiment deserves emphasis. Surviving strips from the papillary muscle of a cat's heart immersed in Ringer's solution at a temperature of 32°C. usually give good spontaneous, rhythmic contractions in 10 to 30 minutes after immersion. Similar strips placed in a solution at a temperature of 37°C. usually give a few strong contractions, but there soon follows a rapid rise in tonus accompanied by a diminution in the extent of contractions which ends with an almost immediate cessation of all activity. This condition is extremely difficult to overcome. The only effective method which we have yet discovered for overcoming this "rigor" is to wait several hours, during which time the temperature is lowered to 32°C. and oxygen is given continuously.

With these exceptions concerning blood, temperature and tension, the technique is essentially that used for smooth muscle strips. Thus, mammalian Ringer's, Tyrode's or Locke's solution[3] may be used. The latter two apparently yield no more satisfactory results than does the former. The pH of the solution was in most experiments close to 7.8. Owing to an unavoidable discrepancy in the colorimetric scale more exact values cannot be given. The solution was, of course, regularly oxygenated.[4]

Even with these precautions in regard to solution, pH, oxygen and temperature, there is generally a quiescent period after setting up a strip before the initiation of rhythmic contractions. The length of the quiescent period varies with the kind of material, with the extent of operation, and with the condition of the muscle strip at the beginning of the experiment. With

[3] Merck Blue Label Chemicals were used in making all solutions.

[4] The oxygen used was that of the Ohio Chemical Co.

surviving cat hearts it is a matter of only a few minutes. With post-mortem material, such as autopsy material or material from the abattoir, the quiescent period may last from two to four hours and occasionally we have seen muscle strips commence spontaneous rhythmic contractions after six hours of absolute quiescence. During most of this quiescent period the muscle gives no indication of irritability and we have found as yet no means of activating it. However, toward the end of the quiescent period there are many ways of stimulating the muscle to give rhythmic contractions.

The usual forms of mechanical stimulation, such as stretching, slightly changing the tension, or direct massage, have been tried. Any one of these may suffice to initiate a brief series of rhythmic contractions. Electrical stimulation frequently is more effective. The usual response of a ventricular muscle strip to such stimulation is a brief series of rhythmic contractions similar to those described by Erlanger for auricular strips.

When a muscle strip is irritable, as evidenced by its response to mechanical or electrical stimulation, yet unable to maintain rhythmic contractions, the effect of adrenalin is very striking. Under these conditions, a single drop of a 1: 1000 solution of adrenalin chloride (P. D. & Co.) added to a beaker containing 50 to 75 cc. of immersion fluid results in the initiation of a series of rhythmic contractions which continue by the hour provided the solution is oxygenated and the temperature maintained at 32°C. The promptness of the response and the character of the initial contractions vary with different strips. Rhythmic contractions occasionally commence within a few seconds. More frequently they will appear nearly half a minute after the administration of the drug and, at times, the response is delayed for two minutes or more. The initial contraction may have a slow rhythm, gradually increasing in rate and extent, or the muscle strip may attain its maximum rhythm after but a few contractions. The important point is that adrenalin not only stimulates the muscle to activity but in some way acts to enable the muscle to maintain its power of rhythmic contractions.

The effect of adrenalin upon the beating strip is by no means as regular or as striking as upon the non-beating strip. Occasionally, especially with a slowly or feebly beating strip, a typical adrenalin augmentation is seen but in the majority of instances there is no change in behavior upon the addition of another drop of adrenalin.

In our experiments adrenalin has proved to be effective on all parts of heart muscle. The tip of the apex, known to contain few, if any, nerve cells, responds to adrenalin. So do all the other ventricular muscle strips. Circular muscle strips have, however, consistently required more adrenalin to initiate contraction than have the papillary muscle preparations. Our inclination, therefore, is to believe that the drug acts as a direct stimulant

to cardiac muscle, rather than upon the neuro-muscular junction in that tissue. This is in accord with the experiments of Eyster and Meek (6) upon the effect of adrenalin on the heart in situ. Abderhalden and Gellhorn (2) also believe adrenalin in small doses acts as a direct stimulant to heart muscle but in larger doses depresses the heart rate through its action on the vagal nerve junctions. Our evidence, however, is not conclusive and requires further investigation.

The fundamental results of our experiments using this simple technique are illustrated by the following protocols:

Protocol 5—Cat.

2:10. Cat given intraperitoneal injection of chloretone
2:30. Deep anesthesia
2:40. Death
3:30. Heart excised—placed in warm Tyrode's solution
3:40. Right ventricle gave slow contractions
3:50. Posterior papillary muscle immersed in Tyrode's solution and a small amount of blood—temperature 31°C.
3:50. Immediate spontaneous contraction. Strong pulsus alternans—contractions shown in figure 1. Weighting lever lessened height of contraction; lever counter-balanced
4:40. Adrenalin—increased force and rate but rhythmicity was of short duration[5]
4:45. Digitalis, 5 drops—revived heart beat
5:15. Contraction weak but present when experiment broken off

Protocol 24—Experiment on human autopsy heart.[6]

Diagnosis: Sudden death at 2:30.
Autopsy, 5:00 p.m.—Finding, arterio-sclerosis.
5:50 p.m. Strip of left ventricle placed in Ringer's solution.
6:30 p.m. Strips set up. Ringer's solution, 50 cc. O_2 given continuously
8:00 p.m. Human blood added to solution—temperature 32°C.
9:00 p.m. Temperature slowly raised from 22°C. to 30°C.
9:30 p.m. Anterior papillary muscle started spontaneous rhythmic contractions —temperature 30°C. Contractions not strong but regular (shown in fig. 2). No response to change in tension, mechanical stimulation, electrical stimulation or adrenalin. Withdrawal of O_2 led to depression of heart beat which improved upon restoral of O_2

The muscle strip continued to beat rhythmically until the experiment was broken off at 12:30 (midnight).

Protocol 44—Cat.

11:00. Operation. Chloroform-ether anesthesia. Blood drawn direct from heart with syringe—promptly defibrinated. Heart excised.
11:20. Strips set up in Ringer's solution and defibrinated blood.

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[5] An adrenalin response of *short* duration was not the usual finding in subsequent experiments.

[6] For the privilege of using this material we wish to express our great indebtedness to Dr. W. H. Watters.

	Anterior papillary muscle	*Posterior papillary muscle*
11:30.	1 drop of adrenalin initiated rhythmic contractions.	Spontaneous contraction—groups of beats at irregular intervals
12:00–12:45.	Temperature gradually raised to 42°C. when contractions ceased. Rise in tonus with rise in temperature	Responded to mechanical stimulation with brief series of rhythmic contractions
1:45.	Temperature 32°C. Rhythmic contractions. Tension released. Adrenalin initiated series of contractions which lasted over 1 hour. Temperature raised to 44°C. Strip still beating	Responded to electrical stimulation with brief series of rhythmic contractions. Adrenalin initiated long-lasting strong series of rhythmic contractions. (This response is shown in fig. 4.) Temperature gradually lowered—contractions abruptly ceased at 24°C. Temperature gradually raised. Rise in tonus with rise in temperature. Strip still beating at 49°C.
5:00.	Both strips still beating when experiment was broken off	

From these protocols it is clear that though there may be a long quiescent period, muscle strips can be made to give spontaneous rhythmic contractions. The quiescent period may be very appreciably shortened by the use of adrenalin. Thus, as soon as the muscle is irritable, the addition of adrenalin to the solution should initiate a lasting series of rhythmic contractions. Moreover, surviving heart strips which begin to pulsate shortly after immersion in solution frequently cease their activity within half an hour. If this happens adrenalin is usually effective in permanently restoring the rhythmicity of the strip.

Protocol 44 illustrates our typical findings in regard to the effect of temperature. Thus provided the muscle strip is allowed to beat rhythmically at 32°C. for at least half an hour, thereafter the temperature range is extraordinarily wide. In this experiment there was a gradual rise in tonus with rise in temperature, but no sharp tonus rise until 46°C. The temperature was promptly lowered and the contractions improved in extent. Later in the afternoon the temperature was again raised. This time the rhythmicity of the strip was not completely destroyed even at 49°C.

From other experiments it is obvious that a sharp tonus rise which results in early "rigor" is more likely to occur in the early part of an experiment than in the later, and is more liable to occur at a high temperature than at a low temperature. Therefore, a low temperature at the beginning of an experiment is recommended.

These protocols show that rhythmicity once established is usually of long duration. Frequently adrenalin is necessary to stabilize contractions and enable the muscle to maintain its power of rhythmic contraction.

In one experiment, however, spontaneous rhythmic contractions initiated at 11 a.m. continued until the experiment was broken off at 5 p.m. without the aid of any stimulation. In several experiments the muscle strip was left in the solution for the night at room temperature without oxygen. Rhythmic contractions were initated the following morning when oxygen was given and the solution heated to 32°C.

Even with every condition favorable, many variations in the behavior of muscle strips may occur. Practically all the variations observed by Abderhalden and Gellhorn (1), (2), (3) in their study of cold-blooded ventricular strips have been observed by us in mammalian ventricular strips. Thus, we have frequently seen pulsus alternans, grouped beats and Lucianic periods, and apparently spontaneous change in rate and in extent of contraction. In spite of the theories based on the syncytial character of the heart muscle it seems to us that marked irregularity in extent of contraction may well be due to the fact that more fibers contract at one time than at another, or that different groups of fibers contract at different times. These variations may be related to those involved in the problem of fibrillation and circus movement (8).

Tonus waves similar to those not infrequently seen in auricular strips from a turtle heart were observed in one experiment. These waves, such as shown in figure 5, occurred at rhythmic intervals, 10 to 15 minutes apart. At the height of the wave the contractions were very rapid and very strong, far stronger than indicated in the record owing to the mechanical difficulty of recording the contractions at the changed height and angle of the writing lever. In this preparation four such tonus waves occurred before the muscle strip steadied down. Then it assumed a distinctly more rapid rate of rhythmic contraction than it had previously shown, i.e., a rate of 81 per minute in contrast to a former rate of 3 per minute.

The cause of variation presents many interesting problems. One of the most important and fundamental of these relates to Purkinje tissue and its effect upon the activity of cardiac muscle. As yet we have not obtained any strips of Purkinje tissue which on sectioning serially contained no heart muscle. The record shown in figure 3 is one taken from the strip of Purkinje tissue of a calf heart—the anterior division of the left branch of the bundle of His. It was approximately a strip 1 cm. long and 3 mm. in diameter. On section it contained a strip of heart muscle, 0.75 mm. in length, 0.25 mm. in breadth, and 0.532 mm. in thickness. It hardly seems possible that this minute amount of cardiac muscle lying at one end in the connective tissue surrounding the Purkinje fibers could be responsible for such pronounced rhythmic contractions. We have, however, subsequently seen very much smaller strips of specialized tissue taken from both the right and left ventricle of the dog's heart give unmistakable spontaneous rhythmic contractions. Histological sections clearly show that

the specialized tissue of the dog heart is much more closely similar to heart muscle and much more intimately related to it than is that of the calf heart. It is correspondingly more difficult to obtain absolutely and unquestionably pure Purkinje tissue from the dog heart than from the calf heart. Indeed, all sections of the specialized tissue of the dog heart so far studied have contained a large proportion of heart muscle. In this connection it is significant to remember that Kolliker (9) in 1854 observed the Purkinje cells of an ox heart contract under the microscope. Further evidence and conclusions must be left to a subsequent report.

SUMMARY. Any small strip of mammalian ventricular muscle (cat, dog, rabbit, sheep or man) can be made to give spontaneous rhythmic contractions without perfusion; even material quiescent for several hours after systemic death may yield strips which will give rhythmic contractions.

Mammalian Ringer's, Tyrode's or Locke's solution may be used as an immersion fluid. From 5 to 10 per cent of defibrinated blood aids in eliciting spontaneous rhythmic contractions, though a high proportion of blood is apparently not advantageous. The optimum temperature is 32° to 35°C.; nevertheless, contractions have been recorded at all temperatures between 24° and 50°C. Marked tension on the writing lever is not advantageous—a counter-balanced lever is recommended. Oxygen is essential for the prolonged continuation of contractions.

In order to stimulate a non-beating muscle strip the usual methods of mechanical and electrical stimulation may be used. These frequently elicit a brief series of rhythmic contractions but rarely a series of long duration. A muscle strip which responds temporarily to electrical stimulation should respond to a potent solution of adrenalin chloride (one drop of 1: 1000 dilution of P.D. preparation) with a lasting series of rhythmic contractions.

The following variations have been observed in papillary muscle preparations; 1, pulsus alternans; 2, grouped beats; 3, "Lucianic periods"; 4, tonus waves.

There is the possibility of applying this method to the study of Purkinje tissue, inasmuch as good contractions have been recorded from strips of

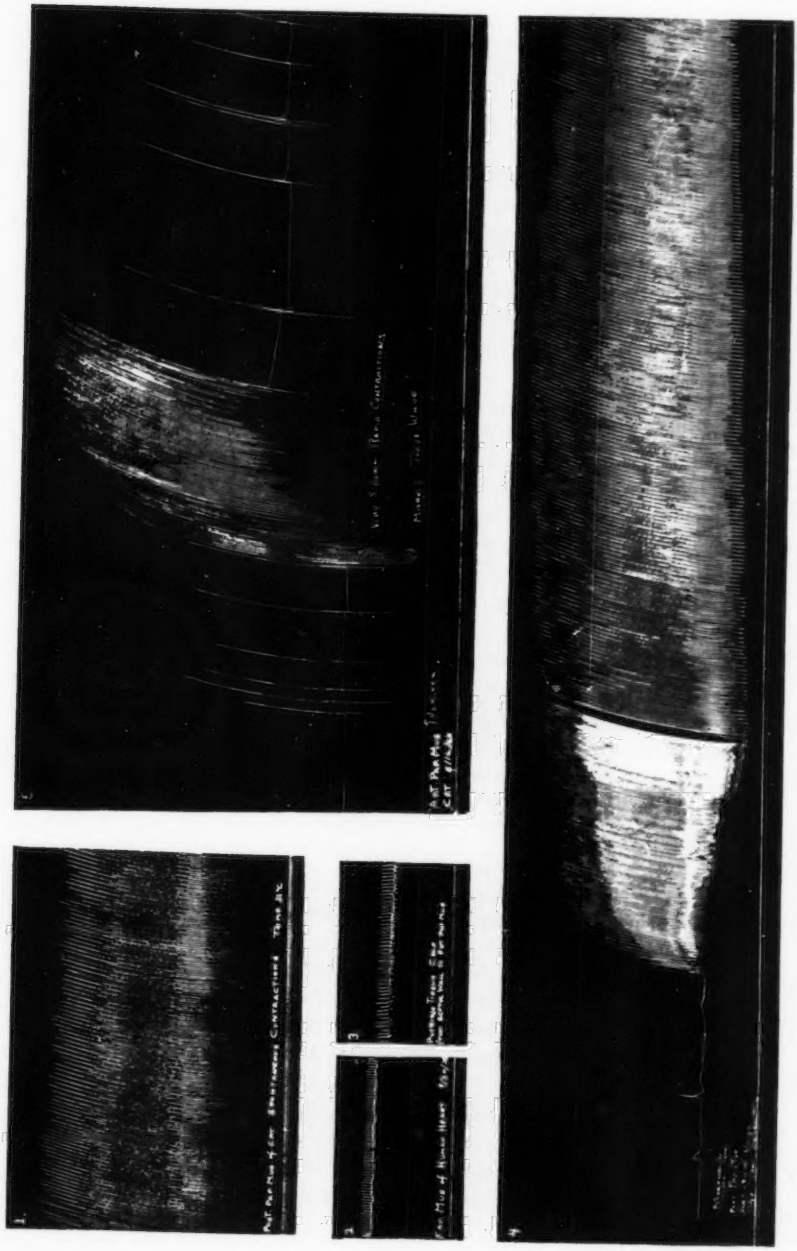
Fig. 1. Spontaneous rhythmic contractions from the posterior papillary muscle of cat heart.

Fig. 2. Spontaneous rhythmic contractions from the papillary muscle of human heart.

Fig. 3. Spontaneous rhythmic contractions from the portion of the Purkinje system which extends from the septal wall to the anterior papillary muscle in the left ventricle of a calf heart. Histological section showed a piece of heart muscle 0.75 mm. in length; 0.25 mm. in breadth and 0.53 mm. in thickness.

Fig. 4. The effect of adrenalin upon an irritable non-beating heart strip.

Fig. 5. Tonus wave—anterior papillary muscle of the left ventricle of cat heart. The time is recorded in one second intervals. Figures reduced one-half.



Purkinje tissue containing a minimum of cardiac tissue. Conclusive evidence concerning the rhythmicity of Purkinje tissue must be left to a subsequent report.

CONCLUSIONS

1. Any muscular strip of the mammalian ventricle has the power of contracting rhythmically when immersed in oxygenated Ringer's solution.
2. The coronary vessels are not necessarily the only source of nutrition of the ventricular muscle.
3. The irregularity in the strength of contractions frequently observed leads us to question the usual conception of the syncytium of the heart muscle in the sense that any impulse once initiated must spread throughout the ventricles.

In conclusion we wish to thank Dr. W. L. Mendenhall for his assistance with technique, and Dr. F. H. Pratt for his many helpful suggestions and his aid in following current literature. Most of all we gratefully acknowledge the inspiration we have received from the valuable suggestions of Dr. A. S. Begg, to whom this work owes its inception. We cannot express too strongly our appreciation of his constant interest and encouragement.

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THE SMALL INTESTINE IN HUNGER

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Received for publication December 26, 1924

Bush (1) in 1862, making observations on a patient with a duodenal fistula, reported that periods of active peristalsis alternating with periods of rest occurred in the intestine without any regularity in the recurrence of the periods of activity.

Boldyreff (2) observed that borborygmi frequently occur during hunger periods and that the free end of a fistula of the intestine underwent changes which he interpreted as being due to movements of the intestine. The frequent occurrence of borborygmi with hunger periods has also been reported by Hertz, Cannon and Carlson (3). We have frequently witnessed it in our work.

Carlson found in dogs with a duodenal fistula that during hunger periods bubbles of gas, fluid, etc., were expelled from the fistula. Carlson has made simultaneous records of the motility of the stomach and colon during hunger and found that contractions of the colon were not correlated with those of the stomach. It has been demonstrated (4), (5), (6) that a tonus rhythm occurs in the lower esophagus and cardia which runs parallel with hunger contractions, except that the esophageal contractions appear to lag (3, p. 76).

Carlson (3) in discussing the rôle of the intestine in hunger admits the possibility that the intestine may contribute to the sensation of hunger, but states that the proof is still wanting. The evidence at hand is obviously very meager and entirely indirect.

METHODS. In this work *a*, three normal men, *b*, four dogs with a Thiry's fistula of the duodenum and *c*, two dogs with a duodeno-esophageal anastomosis and a pouch of the entire stomach have been used.

A special balloon described by Ivy and Vloedman (7) with the exception that the usual duodenal tube was used instead of black rubber tubing. One balloon with tube was passed into the duodenum, the other into the stomach. Water manometers were used.

The position of the balloon in the duodenum was verified in every experiment by use of the fluoroscope. In man we not only used the fluoroscope, but after completing a series of tracings, we further verified the position of the balloon by use of a barium meal.

The Thiry's fistula of the duodenum and jejunum was made by making the proximal incision through the duodenum just caudad (1 inch) to the duct of Santorini and the distal incision from 15 to 18 inches caudad to the proximal incision. The duodenal end of this resected loop was brought to the exterior.

The method for making a pouch of the entire stomach has already been described (8).

Results. *On dogs with a Thiry's fistula.* The Thiry's fistula of the duodenum and jejunum manifests rhythmic segmentations and a tonus rhythm neither of which are related in any way to the contractions of the

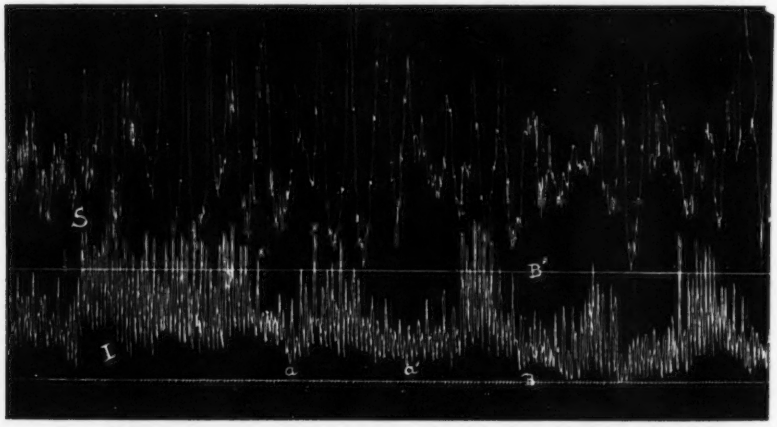

Fig. 1. Simultaneous tracing of the motility of the stomach and a Thiry's fistula of the duodenum and jejunum during hunger. *S*, stomach; *I*, Thiry's fistula. Thirty cubic centimeters air in balloon in stomach; 10 cc. air in balloon in fistula. Note that the rhythmic segmentations, *x*, and tonus rhythm, *a-a'*, are not correlated with the hunger contractions. *B*, base line of intestine; *B'*, base line of stomach.

empty stomach (fig. 1). The gastric hunger motility can be inhibited by food, etc., without affecting the motility of the Thiry's fistula. The tonus rhythm only appears when there is from 10 to 15 cc. of air in the balloon, whereas the rhythmic segmentation can be recorded with as small quantities as from 2.5 to 5 cc. of air in the balloon. Sometimes when periods of type II or III contractions (3) are interposed between type I and II contractions (fig. 1, in part demonstrative) the height of the tonus rhythm in the duodenum is reached just as the period of the type II and III contractions is completed. Since this does not always occur we are reticent in considering it as significant. It is possible, however, that there are times during which the extrinsic reflex centers controlling the tone of the duodenum

are affected by impulses coming from the stomach when showing marked tone.

We have frequently observed inhibition of hunger contractions by distention and mechanical stimulation of the Thiry's fistula of the duodenum and jejunum as reported by other observers (1), (2), (3).

On dogs with duodeno-esophageal anastomosis and a pouch of the entire stomach. In these dogs, the vagi being cut, one would expect the stomach

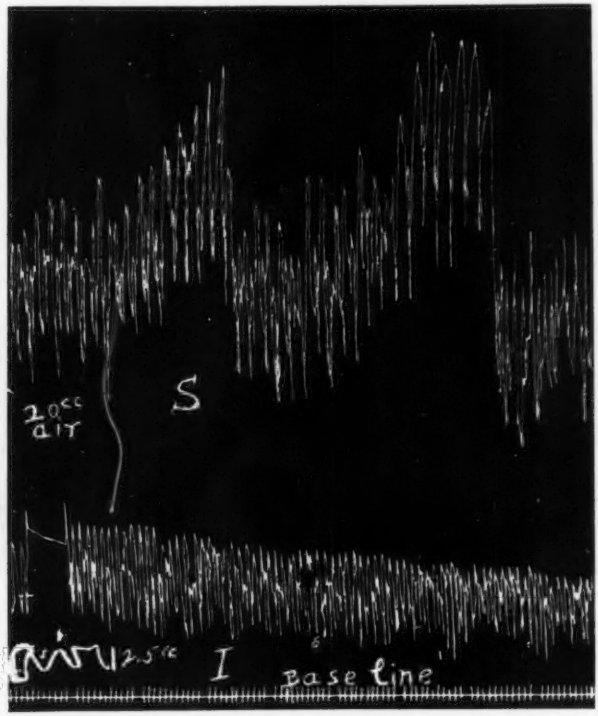

Fig. 2. Simultaneous tracings of the motility of a pouch of the entire stomach, *S*, and of the duodenum, *I*, during hunger in a dog with a duodeno-esophageal anastomosis. Note that there is no relation between the movements in the stomach and duodenum during hunger. The marked tonus shown by the stomach in this tracing is quite characteristic for animals prepared in the manner stated above.

to show a definite reduction in tone. Instead, the stomach of both of the animals shows marked tone on 24 hours' starvation. We do not have, as yet, an adequate explanation for this unexpected observation. The motility and tonus of such a stomach, however, are inhibited when food is ingested, the inhibition occurring via the splanchnic reflex arcs.

When simultaneous tracings are taken of the motility of the stomach and duodenum in this preparation during hunger no relation is observed (fig. 2). The amount of air in and the position of the balloon in the duodenum was varied with the same results. One of the animals was starved for five days with the only result that the tonus of the stomach increased up to the third day. This tonus is practically continuous, except for variations similar to that shown in figure 2.

On man. According to our experience it is more difficult to pass a duodenal tube with a balloon on it into the duodenum than it is to pass the

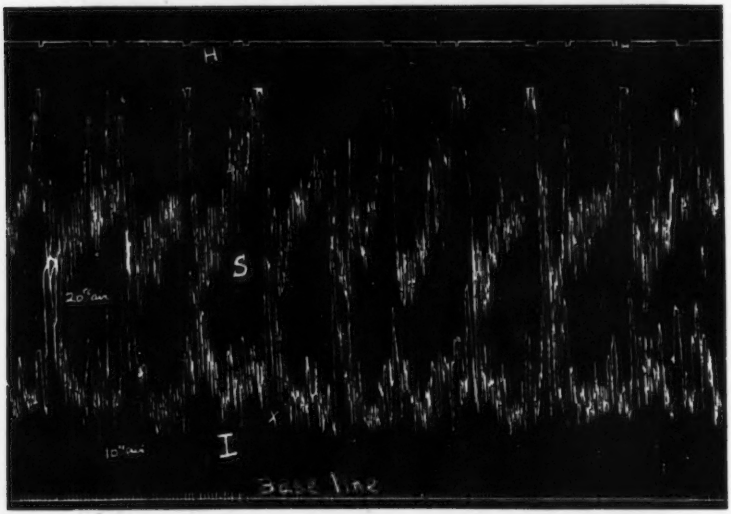

Fig. 3. *Man.* Contractions occur in the duodenum synchronously with hunger contractions in the stomach, the duodenal contractions sometimes, *x*, reaching their height after the hunger contraction has ceased. Balloon in the first six inches of the duodenum. Tracing is lettered as in figure 4.

tube itself. We have more success if the tube and balloon are swallowed the night before. The position of the balloon in the duodenum can be easily proven by use of the fluoroscope accompanied by the introduction of air into the stomach through the stomach tube and into the balloon. Any uncertainty can be settled by taking a barium meal after the experiment and observing that the balloon is outside the stomach, which we did in every instance.

Our tracings show that movements occur in the duodenum either simultaneously with or shortly after the contractions of the stomach. If the tube is in the first four or five inches of the duodenum, the contractions

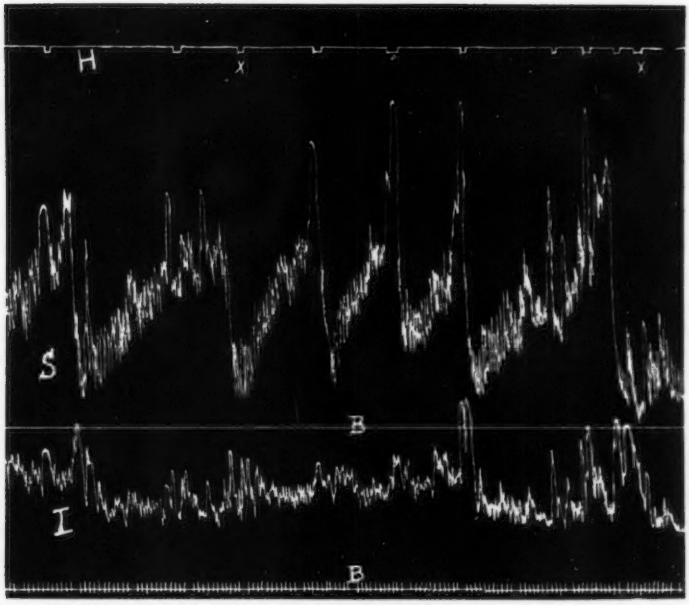

Fig. 4. *Man.* Subject M. *S*, stomach; *I*, intestine; *H*, signal magnet tracing (subject blindfolded) of hunger pains. Note that the hunger sensation, *x*, sometimes occurs after the stomach contraction with the contraction in the duodenum. Tip of balloon in duodenum slightly to the right of the mid-line of the body.

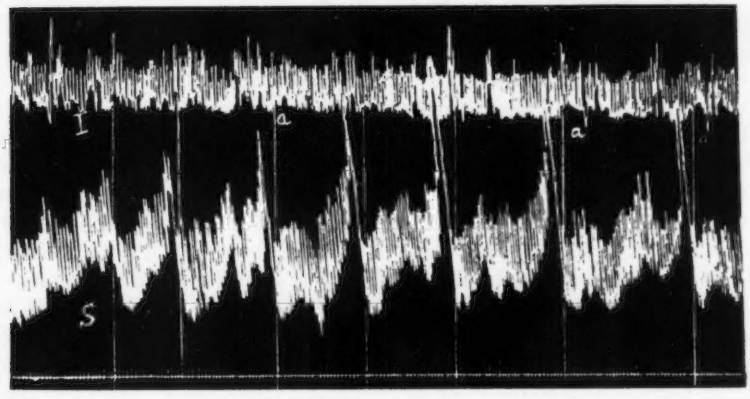

Fig. 5. *Man.* Subject I. Balloon in the duodenum to the left of the midline, the tip being in the jejunum. The tonus wave in the duodenum is just evident and usually occurs after the hunger contraction has disappeared.

never fail to occur and are very marked (fig. 3). The further down in the duodenum the balloon is located the less marked the contractions and the less the occurrence of a contraction associated with the contraction of the stomach. A comparison of figures 3, 4 and 5 demonstrates this phenomenon. If the contractions of the stomach cease due to a rest period, these contractions in the duodenum also disappear.

When the trained subject is blindfolded with a key in circuit with a signal magnet in his hand and is instructed to signal the more intense phase of the hunger pain, it is found that he may signal either prior to, coincident with or just after the height of the contraction in the stomach as shown by the tracing and frequently coincident with a contraction of the first part of the duodenum.

In this work several accessory phenomena were experienced that were interesting.

One subject (I) at the beginning of this work found that when the balloon was in the duodenum, the onset of hunger contractions was delayed and that shortly after the appearance of hunger motility nausea occurred and the balloons were vomited. This did not occur when experiments were resumed several months later. The only explanation that has occurred to us is that the subject's duodenum was more irritable than normal from some unknown cause.

Two subjects (I and V) experienced a referred pain (soreness and tenderness) at the junction of the middle and lateral third of the clavicle covering an area 3 cm. in diameter the day following such an experience as follows: On pulling the tube and balloon from the duodenum it suddenly was caught either at the pylorus or cardia, we believe the former, and marked nausea and vomiting resulted with "doubling up pain" in the epigastrium stopped by drinking water, no pain or distress being observed immediately after removal of the balloons. This same phenomenon was observed by the same subjects sometimes after acid was introduced into the duodenum by duodenal tube. If the tubes are removed slowly, this phenomenon does not occur. It is known that such a referred pain occurs in diaphragmatic pleurisy involving the apex of the diaphragm and when the peritoneal surface of the apex of the diaphragm is stimulated by a wire (9). This fact suggests that the above disturbance might be due to a cardiospasm and traction on the diaphragm. But according to our experience, it occurs just about the time the tip of the tube or balloon, which is enlarged due to the bulb or lead weight, reaches the pyloric orifice. We have also sometimes experienced (subjects I and V), on distention of the duodenal balloon with 50 cc. of air, nausea and a sensation of chilliness. This is frequently observed when acid or sodium bicarbonate is introduced into the duodenum (10), (11).

One subject (I) in our series has never experienced the not unusual

accessory phenomenon of nausea of hunger and headache while taking records of the hunger motility of the stomach only. But this subject while taking hunger motility of the stomach and duodenum in several experiments experienced slight nausea during the hunger periods and headache toward the end of the experiment. The headache disappeared a few minutes after drinking milk. This observation suggests that the presence of the tube in the duodenum decreased the nausea and headache thresholds. It is quite possible, since it is known that irritation of the duodenum provokes nausea and vomiting so readily, that the nausea of hunger is duodenal in origin.

We have also observed by use of the fluoroscope and by a change in the tracing that the balloon in the duodenum may be regurgitated into the stomach during a brief attack of nausea without the occurrence of actual vomiting.

Discussion. Our failure to observe contractions of the duodenum in Thiry's fistula and duodeno-esophageal-anastomosis animals coincident with hunger contractions of the stomach, and our observations in man that such a phenomenon does occur show conclusively, we believe, that the contraction in the duodenum is due only to the passage of the gastric hunger contractions, or their effect, from the stomach down to the duodenum. That is, the motility that the duodenum manifests during hunger is entirely dependent on enteric connections existing between the stomach and duodenum.

The passage of a contraction wave from the stomach over the pyloric sphincter to the duodenum, or the existence of a relation between gastric and duodenal motility during digestion seems to be evident from the observations of Wheelon and Thomas (12). Luckhardt, Phillips and Carlson (13), studying the control of the pylorus report that during digestion the peristaltic waves course toward the pyloric sphincter and on reaching it, relaxation occurs and the dark mass is hastily passed through the entire course of the duodenum. Cole (14), however, states that "the duodenal cap is evacuated by a broad peristaltic wave, independent of the gastric peristalsis, which forces the chyme through the duodenum in 'finger like masses.'" Our fluoroscopic observations on man lead us to believe that in the majority of instances a contraction of the duodenal cap, at least the distal portion of the cap, occurs after a brief pause following the arrival of a gastric peristalsis at the pyloric sphincter.[1] Alvarez (15) who holds that gastric waves stop at the pyloric sphincter grants that some influences pass over the sphincter to start peristaltic rushes in the bowel.

[1] Doctor Orndoff, Roentgenologist, North Chicago Hospital, has stated that in 90 per cent of the patients examined by him the contraction of the "cap" occurs shortly after the arrival of a contraction at the pylorus, which he does not believe to be a coincidence.

Our observations strongly suggest that the motility which the esophagus and cardia manifest during hunger is also dependent on the enteric connection of the esophagus and stomach as it is reported (3), (4, see tracing p. 21) that the movements in the esophagus appear to lag behind the movements of the stomach during the hunger motility of the latter. It appears then that the hunger movement, or its effect upon enteric reflex mechanisms, arising in the stomach passes in both directions along the alimentary canal. So far as we are aware, such an "anakatastaltic" movement of the alimentary canal has not been previously described.[2]

Such a phenomenon would be difficult to explain on the basis of Alvarez's "gradient theory" unless it is assumed that in hunger the metabolic rate of the stomach is raised to such an extent that the "normal gradient" between esophagus and stomach is reversed. If such were the case, at least some esophageal dysphagia should be encountered during a hunger period. We have failed to experience such a dysphagia and know that it is not mentioned in the literature on hunger. It might be objected that the inhibition of hunger that occurs from the mouth reëstablishes the "normal gradient" and hence prevents a dysphagia. But if such a sudden change in metabolic gradient is possible through long reflexes, it argues against a gradient hypothesis playing a basic rôle in the motor activity of the upper portion of the alimentary canal.

Although our tracings show that sometimes a hunger sensation may occur when the contraction in the stomach is on the decline and the contraction in the duodenum is at its height, it does not follow for at least two reasons that the contraction in the duodenum is the cause of some of the hunger pain or sensation. Since it is known that during a hunger pain there may be more than one contraction wave (16) coursing over the stomach, it is quite possible that the last wave is causing the pain that is present during the descent of the gastric tracing or during the time the duodenum shows contractions. Also, since it is well known that an effect, such as pain, outlasts the stimulus, we cannot say that some of the hunger pain comes from the duodenum because it contracts at a time the tracing of the stomach shows relaxation. However, one cannot deny the possibility that the contraction in the duodenum causes some of the hunger sensation, since the duodenum actually contracts during the time the sensation is felt. Only observations on man following total gastrectomy can settle the question. The literature does not contain authenticated or proven cases of such an operation on man, as far as we have been able to ascertain.

It is quite possible that the contractions of the duodenum during hunger

[2] Doctor Carlson states that he has observed ascending and descending contractions in the upper third of the esophagus in a girl that had an esophageal stricture for quite a number of years.

and the latter part of digestion accounts for—in part at least—the intermittent pain associated with duodenal ulcer. Also, since resection of the gut at the pylorus separates the duodenum from the motor drive (digestive and hunger) of the stomach, it seems quite likely that this is one of the factors involved in the favorable results reported on the healing of duodenal ulcers in the combined operations of gastro-enterostomy and pylorectomy. This is analogous to the favorable influence of paralyzing or section of the sphincters on the healing of anal lesions and falls into line with the experimental results showing that manipulation delays the healing of acute lesions of the gastric mucosa (17).

SUMMARY

1. The duodenum during hunger manifests contractions synchronous with those that occur in the stomach with the exception that frequently the duodenal contractions lag behind those in the stomach. The first part of the duodenum manifests more motility during hunger than the last part.

2. The duodenal motility during hunger is dependent on enteric reflex contractions within the stomach.

3. Attention is directed to a new gastro-intestinal motor phenomenon—anakatastalsis—which consists of a spreading of a contraction wave or influence from the site of origin of the gastric hunger contraction upward involving the esophagus and downward involving the duodenum.

4. Several accessory phenomena observed in the course of this work are mentioned and discussed.

5. We cannot conclude that the duodenal motility contributes to the sensation of hunger, but we do cite and discuss some evidence suggesting that the nausea and headache of hunger is duodenal in origin.

6. Our observations as related to duodenal ulcer are discussed.

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STUDIES ON THE MOTILITY OF THE DENERVATED HEIDENHAIN POUCH

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Received for publication December 26, 1924

So far as can be determined, the only observations made on the motility of the Heidenhain pouch are those made by Robbins and Boyd (1), who described what appears to be the fundamental rhythm of the pouch. In one animal these observers attempted to denervate the pouch by stripping the mesentery of the pouch and cauterizing a ring about the vessels and section of all extrinsic nerves accompanying the vessels. This procedure is open to the criticism of trauma to the vessels and resulting irregularities in the circulation to the pouch. In this animal Robbins and Boyd describe the pouch as having some activity but not of the constant regular type as occurred before this operation.

In view of the fact that observations were being made by Doctors Ivy and Lim on the secretion from the denervated Heidenhain pouches it was suggested by Doctor Carlson that the work on the motility of these preparations be extended.

METHODS. The balloon method of recording motility was used. In some of the later experiments simultaneous respiratory tracings were made with a pneumograph. The animals were trained to lie quietly on the table. No anesthetic was used in any case. The animals were operated in the usual manner to make a Heidenhain pouch. Some weeks later the coeliac ganglion was removed and the animals allowed to recover. For details of technic of operation and literature see Lim, Ivy and McCarthy (2).

RESULTS. *1. Spontaneous motor activity* occurs in the denervated pouch. There are two general types of motility which are more or less related and which may be expressions of the same fundamental mechanism. First, *tonus changes* in which there are marked variations in intra-pouch pressures. These are periodic and apparently related to the groups of contractions. In most cases the variations in intra-pouch pressures are a gradual rise and decline. In many cases these seem to be the precursors of the active contractions. Second, *period groups of contractions* occur in the denervated pouch. These are after the general pattern described by Robbins and Boyd (1). It is not known whether the large contractions observed are physiological peristalses or expulsive attempts of the pouch due to the pres-

sure of the balloon in the pouch. At most times when the contractions are present there is a rather definite grouping and periodicity. There is a general relationship between the time of active contractions and the periods of relative inactivity. The time of rest is longer when the period of activity is greatest and most vigorous. This relationship is so striking and so regular in its occurrence as to suggest a refractory mechanism whereby a compensatory "long pause" occurs in relation to the activity. Or, it may be a fatigue effect of the complex neuromuscular mechanism controlling motor activity. The pouch when isolated *in situ* from the central nervous system possesses an automatic activity the true nature of which is not known at present.

2. Stimulation by distention. It has been repeatedly observed that when the balloon is first introduced into the pouch there is a short period of increased motility associated with increased intra-pouch pressure. This seems to be in some way associated with the distention of the pouch coincident with the inflation of the balloon at the beginning of the experiment.

In the relatively quiescent pouch suddenly increasing the pressure within the pouch is generally followed by an immediate increase in tone and a development of contractions. As a rule one or two contractions immediately follow the distention. This stimulation is only temporary. This temporary stimulation does not occur if the main stomach is quiescent due to an inhibition from food. It occurs usually when the main stomach is empty. There are some cases in which addition of air to the balloon in the pouch only modifies the amplitude of the smaller contractions. In some cases it has been noted that decreasing the intra-pouch pressure is followed by a loss of spontaneous motility. This suggests an optimum tension for the spontaneous motility. It is the experience of all workers on isolated preparations of smooth muscle that there must be the proper tension for the best activity. In these preparations there is a rapid failure of activity when there is too much weight counter-balancing the muscle. Also there is no development of activity if there is not enough tension on the muscle. It is generally accepted that distention of the gut stimulates Auerbach's plexus which in turn initiates rhythmic contractions. No stimulation occurs from distention when the pouch is inhibited due to food in the main stomach. It is evident therefore that the neuro-muscular motor mechanism of the pouch is in a condition of altered excitability during the so-called "food inhibition." This condition is present for several hours following ingestion of food.

Lim, Ivy and McCarthy (2) have measured the amount of air necessary to produce stimulation of motility in the main stomach. These observers noted that "when 200 cc. of air was introduced into a balloon in the stomach the intragastric pressure was raised some 10–15 mm. Hg. and motility initiated or increased. A second inflation (200 cc.) one to three hours follow-

ing the first was less effective. This is especially true if the first inflation be a prolonged one."

In a previous communication (3) it was pointed out that the complex neuromuscular motor mechanism is subject to fatigue and conditions simulating the refractory condition of the nervous synapses as suggested by Sherrington (4) for spinal reflex centers.

It is evident that we are here dealing with a complex nervous mechanism but it is important that the element of muscle tension be not disregarded. The elasticity of smooth muscle and its property of responding to the stimulus of tension are of prime importance in all matters of gastro-intestinal tone and motility. The tone and active contractions of any segment of gut are the resultant of three factors: first, muscle tension; second, internal nervous plexuses which are in general governed by the same laws as the spinal reflex centers; third, the extrinsic innervation, when present.

3. Attempts at reflex inhibition of motility. In order to demonstrate the presence or absence of physiologic reflex nervous connections with the pouch the following modes of stimulation were employed: stimulation of the rectum with a test tube brush or distention of the rectum with a balloon, pricking the skin with a needle, stimulation of the mouth and pharynx with a stomach tube, noises in the room such as barking of other dogs, feeding other dogs in the room. In one case the animal was retching and vomited. No inhibition of the denervated pouch occurred from any of these forms of stimulation. In one case it was possible to check on the stimulation of the rectum in an animal with the pouch not denervated. In that case inhibition occurred. However, in one other animal which had not been denervated rectal stimulation was without effect on the pouch motility.

These methods of stimulation were employed because it is generally accepted that when the stomach is in connection with the central nervous system these will cause inhibition of the motility in progress. Not knowing all of the possible paths of the reflexes concerned with the inhibition of the stomach, it is not possible to state that all the extrinsic reflex connections of the pouch are severed by an operation. But the fact still is that the pouch isolated as these have been is not inhibited by the modes of stimulation which usually inhibit the stomach. No experiments were made to test out the effects of sham feeding on pouch motility.

4. Inhibition of pouch motility by food in the main stomach. The experiments of this study have amply confirmed the work of Robbins and Boyd (1) that when food is placed in the main stomach an inhibition of pouch motility promptly occurs. In this study meat was the food most commonly employed and it in the form of chopped steak, etc. Milk was also used. In all cases inhibition promptly occurred when the food was taken (fig. 1*A*).

5. Effect of water in the main stomach. Water in the main stomach usually

has no influence on the motility of the isolated pouch. At times there is a fleeting inhibition in which the next period of contractions is delayed a short time. Water was usually given by stomach tube. In some cases the animals drank it. Varying amounts from 40 to 300 cc. were used.

6. Effect of cold water meat extract in the main stomach. Since it was noted that water at room temperature does not usually cause inhibition when

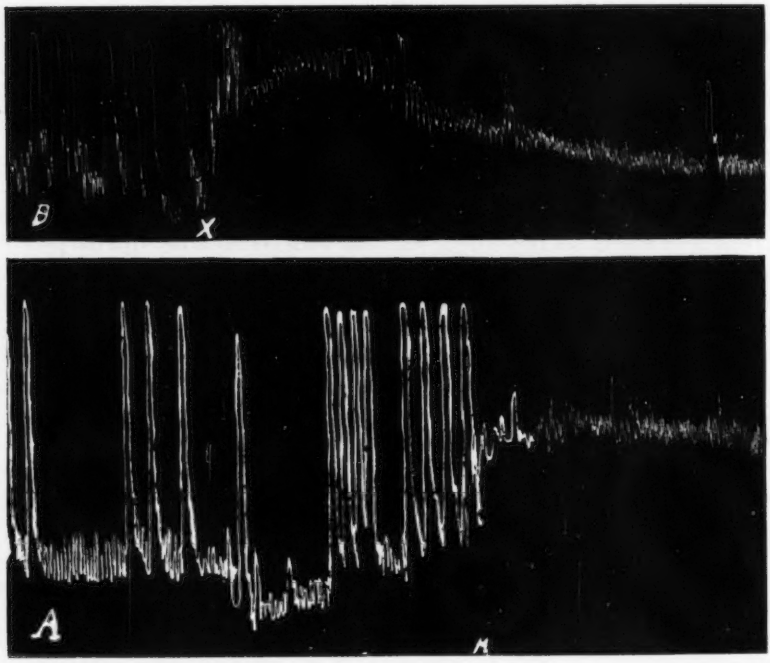

Fig. 1. Dog. Tracing A shows meat inhibition (m.) of hunger motility of a "denervated" Heidenhain pouch, coeliac ganglion intact.

Tracing B shows inhibition of cold water extract of meat (250 cc.) (x) of hunger motility of a denervated Heidenhain pouch, coeliac ganglion intact.

Substances introduced into main stomach.

A water manometer was used in making all tracings.

placed in the main stomach it was thought to be of interest to note the effect of water extract of meat. This was prepared by placing the chopped meat in water at room temperature and allowing it to stand for two or three hours. The water was then drained off and given by stomach tube. Inhibition of pouch motility occurred in every case where this was done (fig. 1*B*). The bulk of water was the same as the bulk of meat. It is

evident therefore that at least one of the inhibitory substances of meat is water soluble and has essentially the same effect as when meat is placed in the stomach. The inhibitory effect from the water extract is not as prolonged as from the meat itself. The fact of this being a cold water extract practically eliminates the element of fat inhibition especially noted by Robbins and Boyd (1).

7. Distention of the main stomach with air. The fact of the inhibition after meat and other foods and also the cold water meat extract suggested that the bulk of these substances and the resultant distention might be a factor in causing inhibition of the pouch. To eliminate this the stomach was distended with air. At first air was passed into the stomach through the stomach tube and the latter quickly withdrawn. In these cases tympany was noted on percussion over the stomach region. This experiment was carried out twice and in each case there was if anything a slight inhibition. In later experiments a soft rubber balloon attached to a small soft rubber tube was passed into the main stomach through the mouth. The animals soon became accustomed to the tube in the mouth and lay quietly with it in place. The balloon in the stomach was distended with varying amounts of air from 100 to 450 cc. The latter amount was equivalent to the water displacement of the meat which the animals usually would eat. It should therefore have the same bulk effect. With the stomach distended in this manner, there is an increase in intra-pouch pressure evidently due to mechanical distention of the stomach pressing on the pouch. In all cases no inhibition followed this mechanical distention of the main stomach.

8. On the action of adrenalin and ergotoxin. There are two experiments in this series on the action of adrenalin. In one of these ergotoxin was used. In one case the animal was operated several weeks before the work on adrenalin was done. At that time the pouch was made and all the extrinsic nerves to the pouch that could be isolated were severed. For technic and details of operation see Lim, Ivy, McCarthy (2). At a later date a second operation was made and the coeliac ganglion removed. This was done to insure complete denervation. Experiments were made on the 7th, 13th and 17th days following ganglionectomy. The results were the same in each case. Adrenalin was given intravenously in doses of 2 cc. of 1/25,000 in normal saline solution. The effect of this amount of saline was controlled.

The action of adrenalin on the pouch was prompt and definite in each case. *There was a rapid sharp rise in tone and gradual relaxation* (fig. 2). A simultaneous respiratory tracing shows no definite changes in the respirations. Following the sharp rise in tone, there is not a rapid fall as in a usual pouch contraction. The period for relaxation of tone is long, requiring several minutes (fig. 2). There is also in most cases a delay in the development of contractions. It is noticeable that even though there is a

sharp rise in tone simulating at first a pouch contraction and also the maintenance of an elevated tone, there is not the development of contractions during the hypertonus. The gradual relaxation and also the lack of contractions during this time suggests an antagonism of two activities. These are stimulating and inhibitory, with the former predominating.

This stimulating action of adrenalin is not dependent on the tone of the preparation. It occurs when adrenalin is injected at the height of a contraction and also at the period of increasing tone prior to a contraction. The same reaction occurs when adrenalin is injected during the inhibition due to meat in the main stomach.

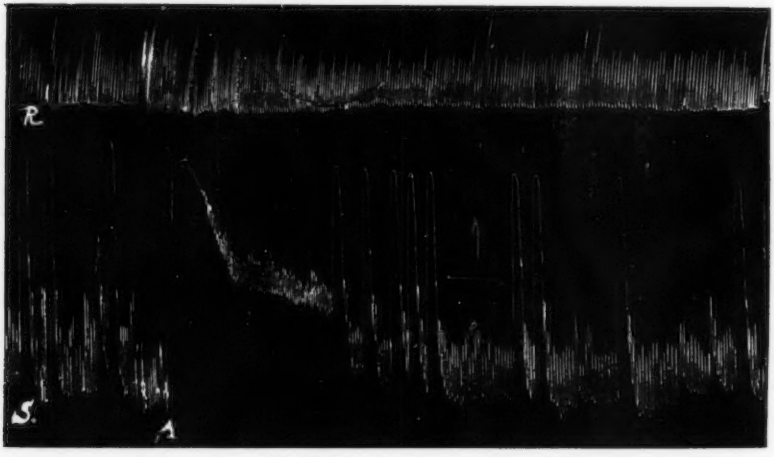

Fig. 2. Dog. This figure shows the effect of 2 cc. of 1:25,000 adrenalin intravenously on the hunger motility of a "denervated" Heidenhain pouch (5 weeks) with the coeliac ganglion removed (11 days). *R* indicates a tracing of respiratory movements.

In the second case the animal was prepared by simply making a Heidenhain pouch. No attempt was made to remove any of the extrinsic nerves which had not been cut during the operation to make the pouch. The animal was given a total of 30 mgm. of ergotoxin (cornutine citrate). Twenty milligrams of this were injected while the animal was showing inhibition of the pouch due to food in the main stomach. Following the injection of ergotoxin there was a slight increase in tone, and the animal vomited a large quantity of food (fig. 3). Subsequent to the vomiting no contractions developed for about 12 hours. Injection of adrenalin subsequent to ergotoxin was followed by a sharp contraction and increased tone without peristalsis (fig. 3).

The question of the length of time of the action of ergotoxin cannot be fully answered at this time. In this experiment, adrenalin was injected subsequently up to 26 days. In each case it was noted that there was an essentially similar action. After 20 days, the reaction to adrenalin was not definite. In most cases there was only a weak, if any, reaction and that suggesting inhibition. In a few cases there was a weak reaction similar to that which occurred immediately after ergotoxin. On the 26th day after ergotoxin it was noted that weak results were obtained. At first there was inhibition, then stimulation, and then again inhibition.

In connection with the work on adrenalin it was repeatedly observed that subsequent injections of adrenalin of the same quantity were followed

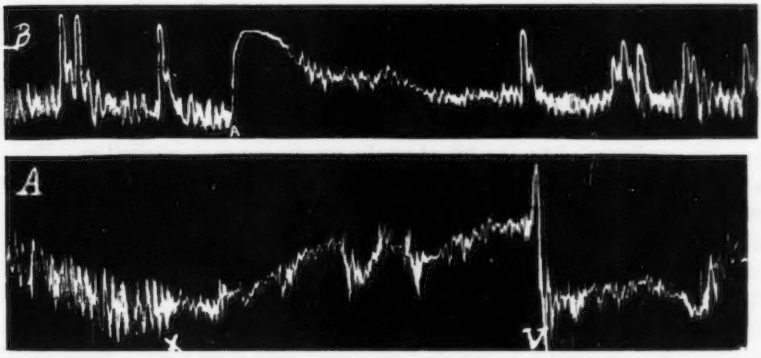

Fig. 3. Dog. Tracing A shows the effect of administration, *x*, of 20 mgm. of ergotoxin (cornutine citrate) in the presence of food inhibition of a Heidenhain pouch colliac ganglion intact. *V* indicates vomiting.

Tracing *B* shows the effect of adrenalin, *A*, (3 cc. of 1:50,000) 12 hours after ergotoxin on the hunger motility of a Heidenhain pouch coeliac ganglion intact.

by a constantly diminishing result. In view of the fact that these animals were being used in another series of experiments and because of the dangers attendant on the over administration of adrenalin it was not thought advisable at this time to determine the amount of adrenalin which might be required before there might be a complete loss of the first effect noted. This may only be a fatigue of the mechanism on which adrenalin acts because of the fact that after a few hours rest the pouch again responds with former vigor to adrenalin in the same quantities.

Discussion of results. The mechanism of the inhibition of the isolated pouch by food in the main stomach has not been determined. Whether there is a reflex mechanism operating to cause the effect cannot be stated now. This much has been determined that the usual methods of causing

reflex inhibition of gastric motility have failed to cause inhibition of the denervated pouch. However, this is not proof positive of the complete absence of any nervous mechanism with possible reflex activities due to impulses from the main stomach. Strongly pointing against the nervous element is the fact that distention of the stomach with a quantity of air equivalent to the bulk of food used does not cause any inhibition of the pouch. Also water in the main stomach will not cause inhibition even when in large enough quantities to give distention similar to the meat.

Increased activity of any organ is usually associated with an increased blood supply to that part. Smooth muscle is usually stimulated during asphyxial conditions. In view of the close relationship of the blood supply to the pouch and the main stomach and the possibility of a hyperemia of the stomach dependent on the presence of food or food extracts it may be that the inhibition of the pouch is associated with or at least in part dependent on a local hyperemia.

Food in the stomach may initiate liberation of some depressive substance either preformed or prepared under the stimulus of food or food extracts and carried by the blood to the pouch causing inhibition. To be sure, the inhibition comes very promptly after food is placed in the main stomach. When meat extract is given by tube the inhibition is already in effect when the tube is withdrawn. On the other hand, Ivy and Vloedman (3) conclude that gastrin in "physiological doses" has no effect on the movements of the stomach and intestine during hunger or the interdigestive period. Also, that hunger contractions are not affected by augmented flow of gastric juice and pancreatic juice that occurs following an injection of gastrin or histamine which is comparable to the amount of secretion resulting from ingestion of a test meal of meat.

No conclusions can be drawn until studies have been made on the blood supply to the pouch and stomach and the effect of artificially produced hyperemia. Crossed circulation experiments should also be made to determine if possible if there is some circulating hormone which is acting as a causal factor in the inhibition.

On the action of adrenalin and ergotoxin. In the work on adrenalin in this series it was assumed that the primary action of adrenalin on the gut is inhibitory. Hoskins (6) has carefully reviewed the literature on the inhibitory action of adrenalin. All observers generally agree on the fact that adrenalin causes inhibition of the gut. A few investigators have called attention to the occasional stimulating action of adrenalin. This is not regular in its occurrence. Boyd (7) has observed an inhibition following adrenalin administration to dogs with Heidenhain pouches which are not denervated. In a previous communication (8) adrenalin was shown to have both stimulating and inhibiting action on the circular fibers of the frog esophagus. It had only an inhibitory action on the longitudinal

fibres. Attention was called at that time to the fact that the primary action of the drug on the neuromuscular mechanism seemed to be dependent on the tonus of the preparations at the time of the injection of the drug. Carlson (9) in his work on the control of the cardia calls attention to both stimulating and inhibitory actions of adrenalin on the cardia and points out the relationship between the tonus and end results of the drug.

Following the administration of ergotoxin, Dale (10) has observed that there is a "reversal" of the usual action ofadrenalin on blood pressure. He called attention to the fact that this was not by producing high tonus, but showed that inhibition still occurred when low blood pressure is present. Brodie and Dixon (11) noted that after painting the stomach of the frog with cocain, there was an apparent "reversal" effect of adrenalin. Dale (12) did not observe any reversal action to adrenalin on the stomach following ergotoxin. His animals were either decerebrate or under anesthesia—all acute experiments. Dale (13) also called attention to the fact that ergotoxin apparently acts by paralysis at the same point where adrenalin stimulation takes place.

In the two experiments in this series there is an apparent "reversal" of the action of adrenalin on the dog's stomach following administration of ergotoxin in one case and after complete denervation in the other. That this is not entirely dependent on the tone of the preparation was shown above by the fact that the reaction occurs at the height of a pouch contraction when the tone is greatest. In this case instead of the usual relaxation of the contraction tone there was an increased tone above the contraction after the injection of adrenalin. There is one isolated experience in this series where adrenalin caused inhibition of the denervated pouch.

It is to be noted that *adrenalin causes essentially the same reaction on the Heidenhain pouch after ergotoxin as it does in the animal which is completely denervated.*

If the point of action of ergotoxin is accepted as worked out by Dale it would appear from the experiments in this series that ergotoxin is selective in the paralysis it causes, thus leaving the motor type of endings only to be acted on by adrenalin. On the other hand, it is not known if ergotoxin in causing the reversal of adrenalin action on the gut is doing so by a pharmacologic antagonism of adrenalin on the inhibitory sympathetic endings or whether the whole effect is due to poisoning and subsequent death of the delicate myoneural sympathetic mechanism. That ergotoxin must do more than cause a simple physiologic block of the sympathetic myoneural junction is shown by the length of time after ergotoxin that the reversal reaction to adrenalin is obtained. If ergotoxin causes permanent paralysis of all sympathetic action it would appear then that the adrenalin action in these experiments was at some point peripheral to the sympathetic

myoneural junction. It may be that it acts on the ganglion cells of Auerbach's plexus and the end effect is the resultant of the stimulating and inhibitory activities of the cells stimulated. The stimulating phase is more prominent. In any case, the fact still remains that in this group of experiments there is a reversal effect of adrenalin following ergotoxin and section of the sympathetic nerves and ganglion.

CONCLUSIONS

1. Spontaneous motor activity in the form of periodic groups of contractions and tonus changes, occurs in denervated Heidenhain pouches.

2. Temporary stimulation of pouch motility occurs when the pouch is distended with air.

3. No inhibition of the motility of the denervated pouch occurs from any of the forms of stimulation (excepting foods), which usually cause inhibition of the gastric motility when the stomach is connected with the central nervous system.

4. Inhibition of the denervated pouch occurs after the ingestion of food into the main stomach. It does not occur when water or air are placed in the main stomach. These were used in quantities equivalent to the bulk of meat used.

5. After ergotoxin and also after complete denervation, intravenous injection of adrenalin is followed by a sharp contraction and rise in tone which is maintained over a period of several minutes with a gradual return to the previous level. There is no development of contractions during this period of hypertonus. In these cases there is a reversal of adrenalin action on the pouch.

The author wishes to express his appreciation for the valuable aid and advice of Doctors Carlson, Lim and Ivy, during the course of these studies.

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A COMPARISON OF THE EFFECTS ON THE ISOLATED BEATING INTESTINE OF CO_2 AND OF A MINERAL ACID

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Received for publication January 5, 1925

In these experiments we were interested in finding out whether the effect, on an isolated beating strip of intestine, of an increase of acidity in the solution surrounding it, is the same whether the increase of acidity is caused by CO_2 or by another acid, no CO_2 being present. In other words, has the CO_2 any specific effect on this preparation, apart from the increased concentration of H-ion which it brings about?

METHOD. We used rabbit intestine. The animal was killed by a blow on the head and bled from the carotids and jugulars. Two or three pieces were taken at random from the ileum and upper jejunum. Part of one of these was carefully cleared of mesentery and two small rings were cut from it, each about 1 cm. in length. The rest of the material was kept in reserve, in Fleisch's solution.

To each of the rings of intestine, two loops of thread were tied, one at either end, and the rings were suspended in the usual way, in separate chambers filled with salt solution, the thread from one end being attached to a rigid arm below and that from the other end hung on a light counterpoised lever above. The levers were of approximately the same magnification and weight and were arranged to write one above another on a moving drum. The two chambers were of about the same capacity (47 cc.) and they were immersed at the same depth in a stirred water bath. This was warmed so as to give a temperature of about 37.8°C. in both chambers which, as comparison showed, were at any time within $\frac{1}{5}$°C. of one another.

The solutions which we used were those recommended by Fleisch (1). A stock solution was made up in the following proportions: NaCl 10.5 grams, KCl 0.5 gram, $CaCl_2$ 0.3 gram, $MgCl_2$ 0.1 gram, H_3PO_4 $\frac{N}{1}$ 5.0 cc., H_2O, 50 cc. This was boiled and filtered. To make the physiological salt solution 50 cc. of stock solution were added to 1 liter of boiled glass-distilled spring water, and either 5.0 cc. of N/1 Na_2CO_3 or, to make the CO_2-free solution which was required, 2.6 cc. N/1 NaOH instead. We

found the pH of the resulting solution as a rule between 7.2 and 7.5 but sometimes when the alkaline N/1 solutions had not been standardized immediately before mixing a fresh quantity, this was found to be too alkaline (pH 7.8 to 8.0). If so, we added a few drops of N/10 HCl to it until the pH was within the desired range.

At the beginning of the experiment compressed air was bubbled through the solutions in both chambers, that to the carbonate-free solution having previously been led through a soda-lime tube to free it from CO_2. The air tube to the carbonate-holding solution was fitted with a T-piece which could be connected with a large spirometer. This spirometer was filled before the beginning of the experiment with air mixed with CO_2 in proportions necessary to give any acidity required for the experiments when bubbled through a sample of the carbonate-holding solution. When the beat of both intestinal strips had become regular, the air to the carbonate-holding solution was clamped off and the air-CO_2 mixture from the spirometer bubbled through instead, weights being placed on the spirometer to give the necessary pressure. From time to time the change in the acidity of the solution caused by the gradual increase of CO_2 dissolved in it was determined by taking one or two samples and reading them by the colorimetric method. We used Clark's (2) color chart as our standard of comparison. The samples required were so frequent and the quantity of solution in each chamber so small, that it was found necessary to use only 1 cc. for each test. This was run into the bottom of a test tube containing about three drops of indicator, great care being taken not to allow the solution to drop through the air and lose more CO_2 than necessary. The readings were taken at once. Comparisons with results from larger samples seemed to show that these small quantities gave results of sufficient accuracy.

While the acidification of the carbonate-holding solution was thus going on, we increased that of the carbonate-free by adding from time to time a few drops of N/10 HCl. Readings of the pH resulting from each such addition were also taken and the changes of acidity in both solutions could thus be made roughly at the same rate.

After the desired acidity of the carbonate-holding solution had been reached and maintained for some minutes, we gradually restored the original hydrogen ion concentration by cutting off the gas mixture and allowing air to bubble through the solution again. A record of the change in pH was kept by taking samples from time to time. While this was going on, we added at intervals a few drops N/10 NaOH to the other solution, testing the pH after each such addition until the original one was restored. The total amount of dilution caused by the addition, first of acid and then of alkali, to this solution was not much more than $\frac{1}{2}$ cc., which may safely be disregarded.

RESULTS. The results of acidifying, whether with CO_2 or with HCl, are to a certain extent the same. As the H-ion concentration increases there is, generally, a slowing of the beat and a reduction of its height and then the beat stops altogether. There is sometimes also a loss of tonus and apparently this occurs about as frequently with one acid as with the other. Out of 22 experiments we had decided loss of tone in 4, and slight loss in 8, acidifying with HCl, and decided loss in 4, and slight in 6, acidifying with CO_2.

In the extent of the acidity, however, which is required to stop the beats altogether there is a great difference between the two acids. Strips in a solution acidified with CO_2 cease beating when this has reached a

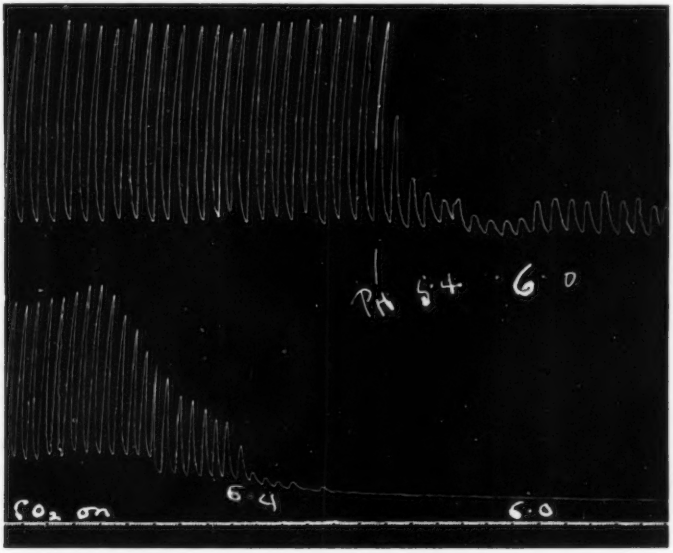

Fig. 1. Effect on beats of acidifying (above) with mineral acid (below) with Co_2.

pH of 6.4 to 6.2 and they do so with astonishing regularity (fig. 1). Out of 22 cases we have only 3 which failed to be completely arrested at pH 6.2. Of these one beat moderately well at pH 6.2, the other two faintly and slowly at pH 6.0 and 5.8 respectively. The fact that the air and CO_2 had to be mixed before the experiment was begun, made it impossible to increase the proportion of CO_2, and consequently the acidity of the solution through which it passed, at a moment's notice, so that we were unable to determine what acidity would have been required to stop the beats of these particular strips completely.

In the case of the preparations in solutions acidified with HCl, we have quite different results. The acidity here is evidently much less effective. Out of all the cases, only one was nearly stopped when the pH reached 6.0, another almost completely at 5.4. The rest continued to beat until the acidity was as high as pH ranging from 5.2 to 4.6 and some were not completely quiescent even then, after exposures of as long as 14 minutes. The beats at this great acidity, however, were nearly always irregular and weak, the height only $\frac{1}{2}$, $\frac{1}{3}$ or even less of that before the acidification (fig. 1). At pH 6.4 the beat is often completely unaffected, quite regular and as high, or almost as high, as at the normal reaction (fig. 2).

The second decided difference between the effect of the two solutions occurs when the normal pH is restored. As the acidity became less in the solutions which contained CO_2 the beats reappeared and this occurred regularly at pH 6.4. They were slow and small at first but gradually became larger and more rapid. In about half the experiments this recovery was complete, the beats, after the hydrogen ion concentration was restored to pH 7 to 7.5, equalling those before the acidification both in height and rate (fig. 3). In the other half of our cases, although the recovery was not complete it was always good, the height being something between 65 and 90 per cent of the original. Only in one case was the height low, 35 per cent.

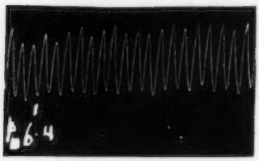

Fig. 2. Beats of strip in solution acidified with HCL, not affected at PH 6–4.

The picture which the strip presents, the beat of which has been stopped with HCl, is quite different. When the original conditions are restored the recovery is not nearly so good. In less than half the cases can one see any improvement at all and in those the height which the beat attains varies between 7 and 75 per cent of the original, generally being between 40 and 60 per cent. There is only one case which shows complete recovery. The beat, too, when it recovers at all from the effects of HCl, is nearly always irregular in shape and the rate a great deal slower than that after CO_2 (fig. 3).

DISCUSSION. It has frequently been found by investigators working on very varied physiological preparations that CO_2 differs in its effects from other acids, either in kind or in degree. We should mention Scott's work (3) on the respiratory center, with its indication of a specific effect of CO_2, that of Clowes and Smith (4) who found it a vastly greater inhibitant of cell growth, segmentation and development than other acids at equal hydrogen ion concentrations, and that of Jacobs (5), who found it to have a specific effect on protozoa and tadpoles. The hypothesis has been advanced that the reason is that the undissociated H_2CO_3 enters the cell more readily than other acids, perhaps on account of its greater

lipoid solubility, and that it there dissociates, increasing the hydrogen ion concentration within the cell. This has been supported by the work of Jacobs (6) on colored flowers, which contained a natural indicator, and on artificial cells. If this explanation be accepted it renders intelligible the results of our experiments. The ready penetration of H_2CO_3 would explain the comparatively low hydrogen ion concentrations at which it is effective and, if one assumes that its effect inside the cell is not a permanently injurious one, its easy escape would account for the good recovery which takes place, once the CO_2 content of the surrounding

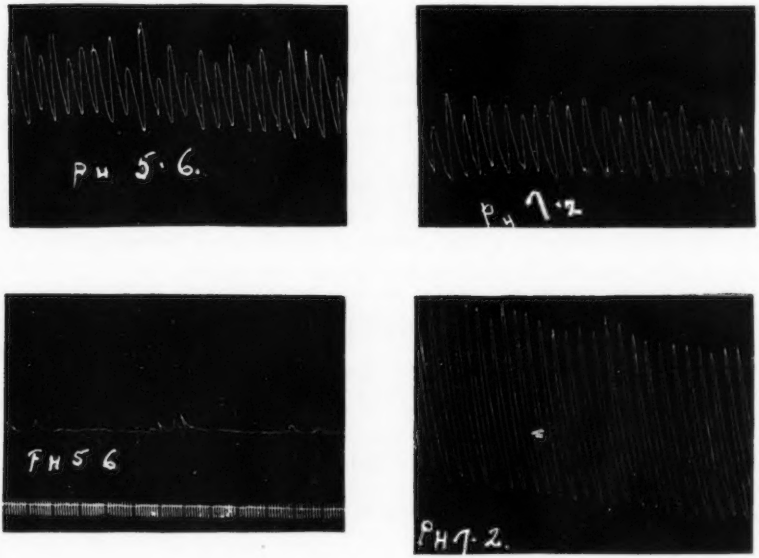

Fig. 3. Effect of reducing acidity produced (above) by HCL (below) by Co_2

solution is lowered again. If the H-ion can penetrate, on the other hand, only by destroying, in part at least, the surface of the cell, the fact that the effect of acidifying in the absence of CO_2 is less prompt, but also more permanent, can be understood.

It is interesting to notice that Gunn and Underhill (7) working on the survival of the beat in isolated pieces of intestine found that as long as the beat lasted at all, though its height decreased, its rate, under constant experimental conditions, was always constant. This was so marked that they suggest "something fundamental in the rate of rhythm." This rate is permanently altered under the conditions of our experiments by

previous exposure to enough acidity to stop the beat, when the acidity is produced by HCl, but not by CO_2.

We wish to offer our thanks to Professor Macleod for his kindness in suggesting the problem and allowing facilities for it, as well as for his advice and criticism in the course of the research.

SUMMARY

1. Working on strips of rabbit intestine beating in an isotonic solution (Fleisch), we have compared the effect of acidifying the solution by dissolving CO_2 in it, with that produced by adding HCl to the solution, no CO_2 being present.

2. In the presence of CO_2, the beats are nearly always suppressed at pH 6.4. In the absence of CO_2, much greater acidity is required to have this effect. The beats in this case are generally unchanged at pH 6.4 and continue in a solution of pH 5.4 or less, although they are then generally irregular and small.

3. When a normal pH is restored the strips which have been exposed to CO_2 generally recover completely both in height, rate and form. The recovery after acidifying in the absence of CO_2 is much less complete, the height being less, the form irregular and the rate slower.

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THE REGULATION OF RENAL ACTIVITY

XI. The Rate of Phosphate Excretion by the Kidney

THE EFFECT OF VARIATION IN THE CONCENTRATION OF PHOSPHATE IN THE PLASMA ON THE RATE OF PHOSPHATE EXCRETION[1]

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Received for publication November 19, 1924

The experiments reported in this paper were planned with the object of determining the effect of changes in the concentration of phosphate in the plasma on the rate of phosphate excretion by the kidney.

In order to induce the greatest possible degree of variation in the plasma phosphate concentration a concentrated solution of Na_2HPO_4 and of NaH_2PO_4 adjusted to a pH of 7.4 was injected into the ear vein of rabbits. During the time over which the relatively high plasma phosphate concentrations thus produced were falling to lower levels successive samples of blood were taken and quantitative collections of urine were obtained by catheter.

On general grounds there is every reason to expect that many other factors besides the concentration of phosphate in the plasma may influence the rate of phosphate excretion although we have no specific information about them except for an experiment recently reported by Haldane, Wigglesworth and Woodrow (1) which showed that the ingestion of large amounts of ammonium chloride was followed by an increase in the rate of phosphate excretion without any appreciable change in plasma phosphate concentration. However, it may be assumed that there are some which accelerate and others which depress the rate and if that is so the optimal conditions for our purpose would be those under which a balance between accelerating and inhibiting factors was maintained throughout the period of time during which observations were being made, since under these conditions the rate would be a function of the plasma phosphate concentration alone. Such an equilibrium was most nearly reached for urea excretion when the external conditions were made as uniform as possible while at the same time the internal conditions were of such a nature as to bring into action the maximum amount of renal secreting tissue. In so far as we could we therefore

[1] This work was aided by the Wellington Gregg Fund for the investigation of Bright's Disease.

tried to duplicate for phosphate excretion the conditions which we had found suitable in studying the effect of changes in blood urea concentration on the rate of urea excretion. The injection of phosphate into the blood stream was in itself, of course, a circumstance which might be expected to put the phosphate excreting activity of the kidney under strain, but in order to produce a diuresis, and to make it more certain that all of the renal tissue had been aroused to full activity before our measurements were begun, we administered 25 cc. per kilo body weight of a 25 per cent solution of urea by stomach tube 2 hours, and 25 cc. per kilo of water half an hour, before the intravenous injection of phosphate was given. We should have liked to allow an interval of time to elapse between the injection of the phosphate and the commencement of the urine and blood collections but we found that the phosphate concentration of the plasma fell so rapidly that we could not observe the effect of high phosphate concentrations unless we started the collections soon after the injection. In most cases therefore the bladder was catheterized and washed out within a few minutes of giving the phosphate. We were obliged to give the phosphate intravenously because we were not able to induce an adequate degree of variation in the concentration in the plasma by the oral administration of phosphate. Tetany followed the injection in rabbits 16, 17 and 18. The details of these experiments are given in table 1 but the data obtained from these animals are not included in our consideration of the question of the relation between the rate of phosphate excretion and the phosphate concentration of the plasma. As an additional precaution no food was given for about 17 hours before each experiment although water was left in the cages. An illustration of the method is given in the following protocol:

Protocol, rabbit 1. Weight = 2150 grams. August 25, 1922. Placed in a separate cage with water but no food during the afternoon of August 24.

9:15 a.m. Given 54 cc. 4 per cent urea solution by stomach tube.
10:45 a.m. Given 54 cc. water by stomach tube.
11:58 a.m. Intravenous injection of 30 per cent neutral sodium phosphate solution in an amount equivalent to 75 mgm. P per kilo body weight.
12:05 p.m. Washing of bladder after catheterization completed.
12:07 p.m. Blood collected. Plasma phosphate = 27.1 mgm. P per 100 cc.
12:19 p.m. Bladder catheterized and washed out. Urine contained phosphate equivalent to 18.4 mgm. P.
12:28 p.m. Blood collected. Plasma phosphate = 18.7 mgm. P per 100 cc.
12:33 p.m. Bladder catheterized and washed out. Urine contained phosphate equivalent to 14.9 mgm. P.
12:48 p.m. Bladder catheterized and washed out. Urine contained phosphate equivalent to 12:4 mgm. P.
12:54 p.m. Blood collected. Plasma phosphate = 13.2 mgm. P per 100 cc.
1:03 p.m. Bladder catheterized and washed out. Urine contained phosphate equivalent to 7.2 mgm. P per 100 cc.

TABLE 1

RABBIT	AMOUNT OF PHOSPHATE INJECTED—MGM. P PER KILO BODY WEIGHT	TIME FROM INJECTION TO MID-POINT OF URINE COLLECTION	RATE OF EXCRETION—MGM. P PER KILO PER HOUR	PLASMA CONCENTRATION MGM. P PER 100 CC.	RATIO: $\frac{\text{RATE OF EXCRETION}}{\text{PLASMA CONCENTRATION}}$
		minutes			
1	75	13	36.7	24.7	1.49
		27	29.7	19.4	1.53
		42	23.1	15.7	1.47
		57	13.4	13.1	1.02
2	50	80	3.7	11.6	0.32
		140	1.6	10.8	0.15
		200	1.7	10.0	0.17
3	47	30	16.3	19.0	0.86
		85	4.6	12.7	0.36
		145	2.4	10.6	0.23
3	0		0.26	5.3	0.05
4	75	88	15.1	15.8	0.96
		150	10.0	11.8	0.85
5	50 and 50 (81 min. later)	44	19.0	15.4	1.23
		109	21.8	20.0	1.09
5	50	35	13.5	13.2	1.02
		93	5.0	9.1	0.55
5	25	42	11.0	9.6	1.15
		104	4.1	7.1	0.57
5	0		0.43	5.5	0.08
6	25	11	10.7	12.9	0.83
		27	4.2	10.3	0.41
		42	2.4	9.6	0.25
		57	1.5	9.3	0.16
7	25	11	12.2	11.0	1.11
		26	6.1	9.4	0.65
		42	4.2	8.3	0.51
		57	4.1	7.7	0.53
8	50	85	4.6	8.7	0.53
		145	3.2	7.4	0.43
		205	2.3	6.5	0.35
9	75	35	26.4	13.9	1.90

TABLE 1—*Continued*

RABBIT	AMOUNT OF PHOSPHATE INJECTED—MGM. P PER KILO BODY WEIGHT	TIME FROM INJECTION TO MIDPOINT OF URINE COLLECTION	RATE OF EXCRETION—MGM. P PER KILO PER HOUR	PLASMA CONCENTRATION MGM. P PER 100 CC.	RATIO: RATE OF EXCRETION / PLASMA CONCENTRATION
		minutes			
9	50	41	15.7	13.9	1.13
		99	5.1	9.3	0.55
		157	4.5	7.5	0.60
9	25	37	9.2	7.7	1.20
		95	3.2	4.9	0.65
9	0		1.8	3.9	0.46
10	50	36	20.9	13.8	1.52
		96	4.5	9.3	0.48
		157	1.6	8.3	0.19
10	25	35	8.9	10.7	0.83
		94	2.2	8.0	0.28
10	0		1.6	4.9	0.33
11	50	40	18.6	13.2	1.41
		97	4.0	9.1	0.44
		157	1.3	7.6	0.17
11	25	34	13.3	11.8	1.13
		95	3.3	7.6	0.43
11	0		0.7	5.4	0.13
12	0		1.2	5.8	0.21
13	50	6	38.5	25.4	1.52
		14	25.9	21.2	1.22
		23	13.8	18.2	0.76
		32	8.6	15.9	0.54
14	0		0.18	4.1	0.44
15	50	12	26.7	18.9	1.41
		26	20.0	14.6	1.37
		42	17.3	11.8	1.47
		56	13.5	10.1	1.34
15	0		0.29	4.1	0.07

TABLE 1—*Concluded*

RABBIT	AMOUNT OF PHOSPHATE INJECTED—MGM. P PER KILO BODY WEIGHT	TIME FROM INJECTION TO MID-POINT OF URINE COLLECTION	RATE OF EXCRETION—MGM. P PER KILO PER HOUR	PLASMA CONCENTRATION MGM. P PER 100 CC.	RATIO: $\frac{\text{RATE OF EXCRETION}}{\text{PLASMA CONCENTRATION}}$
		minutes			
16*	150	37	37.2	30.0	1.24
		97	5.2	16.3	0.32
		157	2.8	14.0	0.20
17†	50	21	15.0	17.8	0.84
		36	11.6	14.8	0.78
		51	8.3	12.7	0.65
		66	5.3	11.0	0.48
18‡	100 and 100 (97 min. later)	46	33.2	24.5	1.36
		137	23.6	33.0	0.72

* Immediately after the injection there was marked difficulty in breathing. The front legs were held stiffly back along the body, so that the animal lay on his chest with the head thrown back. Died some hours later.

† Convulsions occurred immediately after the injection and lasted for about three minutes. No symptoms thereafter.

‡ Recurrent convulsions with weakness, tremor and twitching of the muscles in the intervals. Died some hours later.

The times for the urine collections were taken from the moment of recovery of the last portion of water used in washing out the bladder, and the times for the blood collections were taken as the middle of the short period of time needed to obtain sufficient blood from the ear. The P values for urine and blood were plotted against time, and after a smooth curve had been drawn through the plasma concentration values the average concentration for each period of urine collection was found by averaging ordinates. The phosphate in the urine specimens was calculated as P per hour per kilo body weight.

The details of all the experiments are given in table 1. In the third column the interval of time between the injection of phosphate and the mid-point of each successive period of urine collection has been noted since this turned out to have a bearing on the interpretation of the data. It will be observed that the only important deviation from the procedure followed in the foregoing protocol is the omission of the phosphate injection in certain experiments. This was done for the purpose of obtaining some rates at low plasma phosphate concentrations. The same amounts of urea and water were given in these as in the other experiments. Urine was collected over an hour and samples of blood were taken at the beginning and at the end of the period of urine collection.

Some time before the work was begun Myers and Shevky in this laboratory had investigated the applicability of the Bell and Doisy phosphate method for the purpose we had in mind, and it was this method with the modifications which Myers and Shevky (2) showed were necessary, which was used for the urine as well as for the plasma determinations.

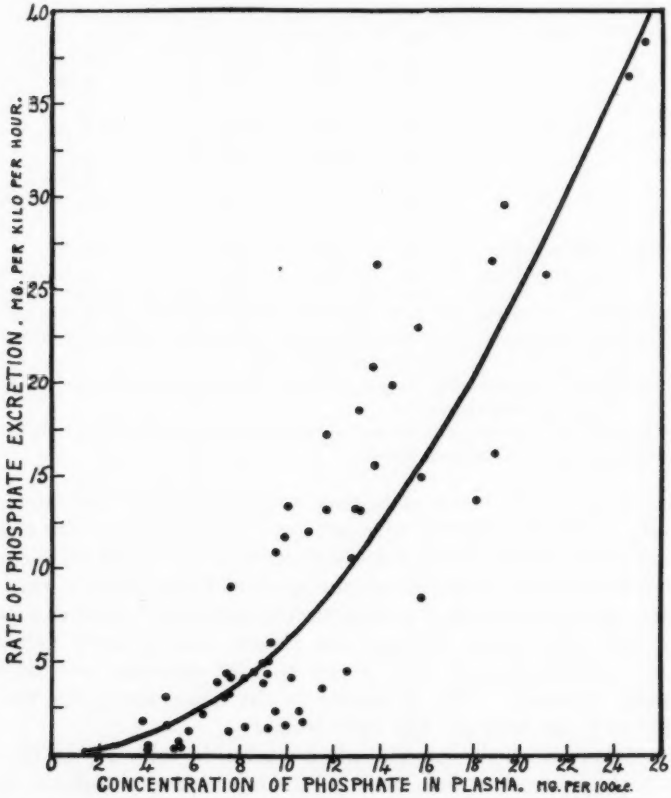

Fig. 1. All the observed rates of phosphate excretion plotted against their respective plasma phosphate concentrations.

In figure 1 all of the observed rates have been plotted against their respective plasma concentrations. Table 2 gives the averages of the groups obtained when the measurements are arranged in accordance with the level of plasma phosphate concentration. The logarithms of these averages are charted in figure 2.

It is shown in figure 2 that the logarithms of the average rates and

concentrations fall along a straight line which would cross an extension of the ordinate scale at -1.2 and which has a slope such that for every increment of change in log concentrations there is twice that amount of change in log rate. In the formula for a straight line, $y = ax + b$, if y represents log rate and x log concentration a thus has the value of 2,

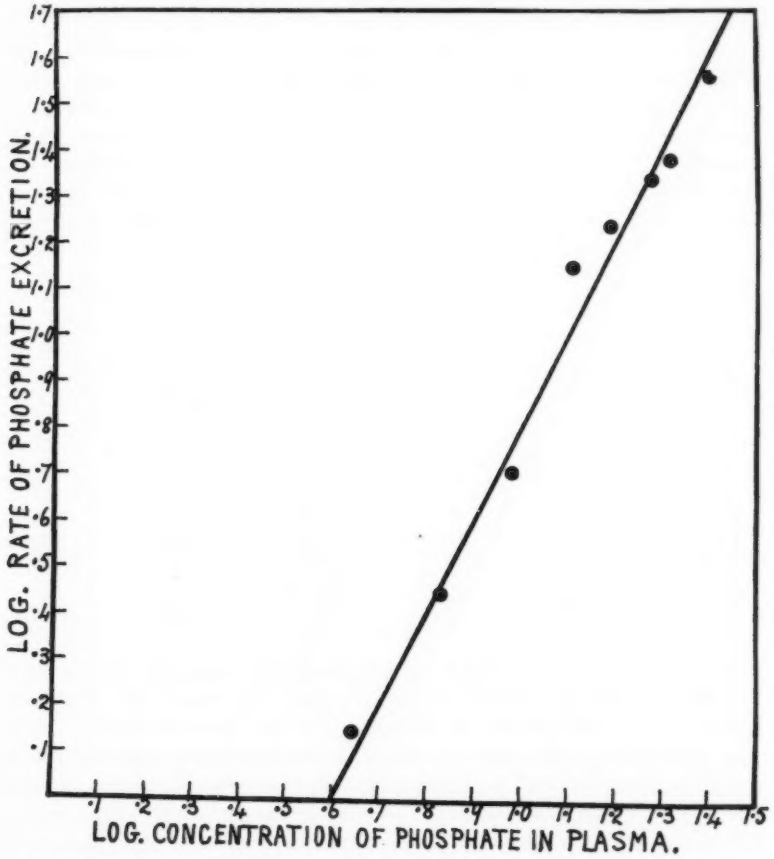

Fig. 2. The logarithms of the average rates of phosphate excretion plotted against the corresponding logarithms of the average plasma concentrations.

and b the value -1.2. The linear nature of the graph of the logs and its two to one slope indicate that the rate varies as the second power of the plasma phosphate concentration. The intersection at -1.2 from the origin indicates a constant factor equivalent to 0.063. The rate of phosphate excretion per kilogram body weight is therefore equal to

0.063 times the square of the plasma phosphate concentration. The curve which has been drawn through the plotted data in figure 1 was constructed from this equation. The coefficient of correlation, r, between the observed and the calculated rates was found to be 0.89 $\pm$ 0.02, a degree of agreement close enough to indicate that this expression of the relation between rate and plasma concentration is a fairly satisfactory summary of our experience.

Does the fact that under the conditions of our experiments the rate tends to vary as the square of the plasma phosphate concentration have any general significance, or is it true only for the special conditions observed in our particular experiments? If we were sure that we had really succeeded in eliminating all variables except the plasma phosphate concentration we might be justified in supposing that the rate would always vary as the square of the plasma phosphate concentration even under

TABLE 2

Average obtained after classification of the data in accordance with the level of plasma phosphate concentration

LEVEL OF PLASMA CONCENTRATION—MGM. P PER 100 CC.	NUMBER OF OBSERVATIONS	AVERAGE PLASMA CONCENTRATION—MGM. P PER 100 CC.	AVERAGE RATE OF EXCRETION—MGM. P PER KILO PER HOUR	LOG PLASMA CONCENTRATION—MGM. P PER 100 CC.	LOG RATE OF EXCRETION—MGM. P PER KILO PER HOUR
2.0– 4.9	5	4.37	1.40	0.641	0.146
5.0– 7.9	12	6.74	2.79	0.829	0.446
8.0–10.9	19	9.55	5.08	0.980	0.706
11.0–13.9	12	12.81	14.01	1.107	1.146
14.0–16.9	5	15.48	17.16	1.190	1.235
17.0–19.9	4	18.87	21.61	1.276	1.335
20.0–22.9	2	20.60	23.84	1.314	1.377
23.0–25.9	2	25.05	37.57	1.399	1.575

inconstant conditions in which the effect of other changing factors might entirely obscure this underlying relationship. Of course the wide scattering of the observations on either side of the curve in figure 1 shows that other factors than plasma phosphate concentration were influencing our rates, but it is still possible that these fluctuations above and below the line were due to causes of an accidental nature, which might neutralize one another, leaving the curve a true expression of the relation between rate and concentration. On the other hand if it happened that the rates observed at the higher levels of phosphate concentration were accelerated by a factor which was not operative at lower levels of phosphate concentration the relationship we found would be true only for this special combination of diverse conditions and would have no general validity.

Now it is, as a matter of fact, quite possible that we have to deal with

just such a situation. It will be remembered that in certain experiments the injection of phosphate solution was omitted in order that we might obtain some rates at low levels of plasma phosphate concentration. But if the injection of phosphate had an accelerating effect on the rate over and above the effect it exerts by virtue of increasing the plasma phos-

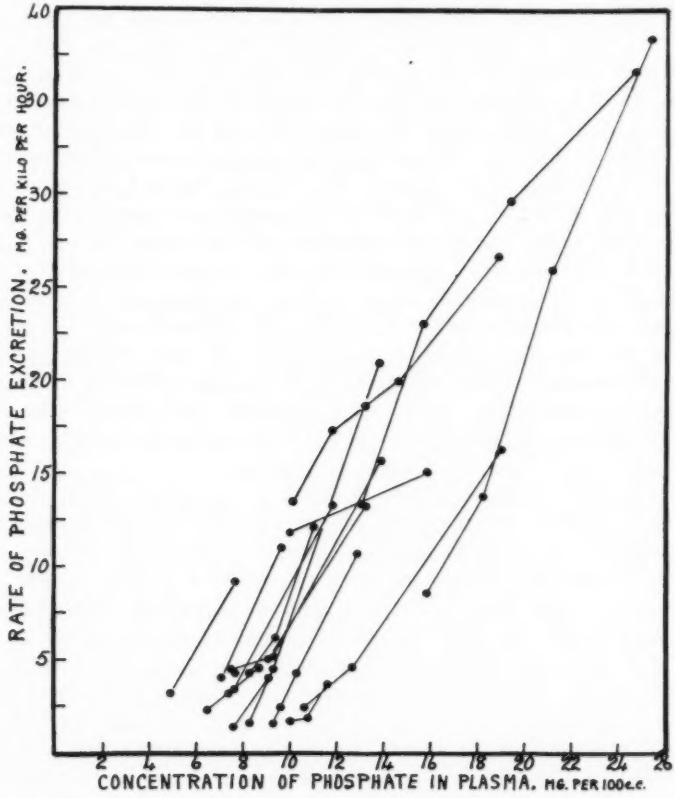

Fig. 3. Shows only the observations made after the injection of phosphate. The rates observed in each separate experiment are connected by lines.

phate concentration the slope of the curve at the higher levels of concentration would be altered while at the lower levels it would remain unchanged. In view of this possibility it seems advisable to consider first of all only the data of the experiments in which phosphate was given. The results have been plotted in figure 3. The measurements made in each separate experiment have been connected by lines.

The average relationship between rate and concentration which might be deduced from figure 3 is more nearly linear than curved. This is especially indicated from a consideration of the individual experiments, for most of the lines tend to be straight, and to be roughly parallel with one another. If we turn now to the eight observations made without the injection of phosphate (table 1) we find that it is impossible in these few observations to see any relationship between the rate and the concentration. The range of concentration covered is only 2 mgm. per 100 cc. of plasma and relatively to one another the rates are very variable. But they have this in common that they lie well to the left of the extension of the average line which might be drawn through the phosphate experiments shown in figure 3. It is of course this lack of concordance between the two sets of data which produces the curve in figure 1, and since the disparity may have been the result of the operation of some factor which was operative under one set of conditions and not under the other, the curve need not necessarily represent the true relation between rate and concentration. This possible source of error may however be avoided if we confine our attention to the measurements obtained under the conditions of the phosphate injection experiments. It may still be possible to find an expression which will apply to all possible variations in plasma phosphate concentration, for in some instances the concentration fell after the injection of phosphate to as low or almost as low a level at that which may exist when no phosphate is given.

An inspection of figure 3 indicates that an extension of the average line which might be drawn through the data would cross the abscissa not at its origin but at a concentration of about 7.5 mgm. P per 100 cc. The equation for the line is therefore not $y = ax$, but $y = ax + b$, and this circumstance might lead us to infer that at phosphate concentrations appreciably below 7.5 mgm. we should find no phosphate at all in the urine, if it were not for the fact that phosphate was never absent from the urines of the rabbits which received no phosphate injection even when the plasma phosphate concentration was as low as 3.9 mgm. per 100 cc. In these observations, however, the plasma phosphate concentrations do not fall to unusually low levels and they are so few in number that they cannot be regarded as at all convincing evidence against the existence of a phosphate "threshold," quite apart from the possibility that the average threshold value might vary under different conditions and might conceivably be lower when only urea and water were given than when the plasma phosphate concentration had fallen after phosphate injection. A much more valid reason for caution in accepting the idea of a renal threshold for phosphate simply on the ground of such evidence as is given in figure 3, is the failure of those who have tried to demonstrate its existence by means of experiments in which the conditions were spe-

cifically designed to induce the excretion of a phosphate free urine. Bertram (3) long ago published a very extensive series of analyses of the urine of a goat on diets which were such as to lead to a marked decrease in phosphate excretion by the kidney. The addition of calcium carbonate to the food was found to still further lower phosphate excretion, but in spite of this some small amount of phosphate was always present in the urine. Within the last few months Blatherwick, Bell and Hill (4) have reported observations on the rate of phosphate excretion in human subjects under the influence of large doses of insulin. In one case the plasma phosphate concentration was reduced to the unusually low level of 1.86 mgm. per 100 cc., but even then some phosphate was excreted by the kidney. The strongly alkaline urine of herbivorous animals has long been known to contain relatively little phosphate, but in looking over the protocols of experiments on sheep, cows, goats, horses and rabbits we have failed to find an instance in which the urine did not contain some phosphate. In our view this constitutes very strong evidence indeed against the conception that there is any threshold for phosphate excretion.

But though we are thus debarred from accepting the hypothesis of the existence of a threshold for phosphate on any but the most direct evidence, the fact that extensions of the lines in figure 3 would cross the abscissa at points well to the right of its origin remains to be accounted for. It appears to us that the explanation may be derived by analogy from a similar experience met with in a study of the factors which influence the rate of urea excretion. In 1917 Addis and Watanabe (5) published a graph of rates of urea excretion measured at varying blood urea concentrations. The curve in that graph has a form very like the phosphate curve in figure 1. It was recognized at that time that the departure from a straight line relationship might be a result of the combination of data obtained under diverse conditions, since some of the rates were observed at varying time intervals after the administration of urea, while others were not. For this reason the measurements made after taking urea were distinguished in the graph from those in which no urea was given. Now an inspection of the post-urea rates indicates that their relationship to the blood urea concentration is best expressed as a straight line which when extented crosses the abscissa at a concentration of about 23 mgm. per 100 cc., a circumstance which might have inclined an observer who had no other facts at his disposal toward the supposition that there was a threshold for urea at or about that blood urea concentration. An experiment which was carried out later but was not reported, showed us how it comes about that a plot of urea rates may sometimes seem to demonstrate the existence of a threshold which other facts prove to be actually non-existent. A group of 14 normal human subjects each took

30 grams of urea in a liter of water in the morning before any food had been taken. Collections of blood and urine were made just before and at every half-hour after the urea had been taken. The urine was voided into graduated cylinders so that the approximate volumes could be read at once, and diuresis was maintained by the administration of the same amount of water as was excreted at each period. The average results are given in table 3.

It is evident from the figures given in table 3 that the relationship between the rate of urea excretion and the blood urea concentration is inconstant and varies with the time elapsing between the taking of urea and the measurement of the rate. After 45 minutes for instance more urea is excreted with a blood urea concentration of 74 mgm. per 100 cc. than is eliminated at 135 minutes when the blood urea concentration is

TABLE 3

Effect on the ratio: $\frac{\text{rate of urea excretion}}{\text{blood urea concentration}}$ *of the time elapsing between the oral administration of urea and the measurement of the ratio*

TIME FROM ADMINISTRATION OF UREA TO MID-POINT OF PERIOD OF URINE COLLECTION	NUMBER OF OBSERVATIONS	RATIO: $\frac{\text{RATE OF EXCRETION}}{\text{BLOOD CONCENTRATION}}$	RATE OF EXCRETION—MGM. UREA PER HOUR	BLOOD CONCENTRATION—MGM. UREA PER 100 CC.
minutes				
15	14	37.5	1834	50
45	14	56.1	4120	74
75	14	48.5	3950	82
105	14	47.1	3662	79
135	14	46.2	3451	76

76 mgm. per 100 cc. The ratio $\frac{\text{urea in 1 hour's urine}}{\text{urea in 100 cc. of blood}}$ indicates an increase in the urea excreting activity of the kidney up to 45 minutes and then a decrease which becomes more and more gradual. Subsequent work showed that constancy was usually not attained until two and a half hours had elapsed since the urea was taken. In this instance therefore we are confronted with conditions which, though uniform externally, have set in action some internal mechanism through which a new and varying factor which influences the rate of urea excretion has been introduced. If the effect of the changing incidence of this factor is overlooked and it is assumed that the conditions are constant, quite erroneous conclusions in regard to the general relation between rate and concentration will be reached. When for instance the rates observed at 15 minutes and at 45 minutes after the administration of urea are plotted against their respective blood urea concentrations, as in figure 4, we obtain a graph

which indicates a threshold for urea at a blood urea concentration of about 30 mgm. per 100 cc. (fig. 4).

The phosphate rates in figure 3 and the urea rates in figure 4 were both determined immediately after the administration of phosphate and of urea respectively. Both graphs indicate thresholds which in actual fact do not exist. But in the case of urea we know that this appearance is the result of an inconstancy in the experimental conditions. For Addis and Drury (6) have recently demonstrated that whenever the conditions are constant the data comply with the equation $y = ax$, y being the rate of urea excretion, x the blood urea concentration and a a constant under

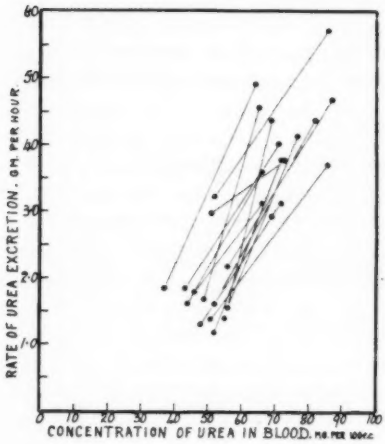

Fig. 4

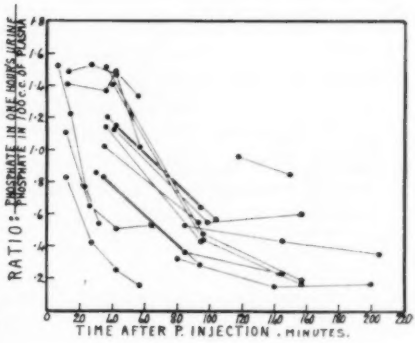

Fig. 5

Fig. 4. Rates of urea excretion measured 15 minutes and 45 minutes after the oral administration of urea plotted against their respective blood urea concentrations.

Fig. 5. Shows the effect on the ratio: $\frac{\text{rate of phosphate excretion}}{\text{plasma phosphate concentration}}$ of the time elapsing between the injection of phosphate and the measurement of the ratio.

appropriate conditions whose value is dependent on the balance which is being maintained between those factors which accelerate and those which depress the rate of urea excretion. In other words, it was found that whenever the conditions were constant the line which represented the relation between rate and blood urea concentration always pointed toward the origin of the abscissa, though the slope of the line might vary under different constant conditions. It is only when the conditions are inconstant and of such a nature that the value of a is changing while the measurements are being made that an extension of the line of relationship fails to meet the intersection of ordinate and abscissa and

is expressed by the formula $y = ax + b$, as in the instance of the data plotted in figure 4.

In the case of phosphate excretion it is possible that the intravenous injection of sodium phosphate may have been a factor which accelerated the rate of phosphate excretion independently of the effect of the increased concentration of phosphate in the plasma, just as the injection of urea increased the rate of urea excretion in a manner which cannot be accounted for on the basis of the change in blood urea concentration alone. If that were so we should expect to find a relation between the time elapsing between the injection of the phosphate and the magnitude of the ratio: $\frac{\text{phosphate in 1 hour's urine}}{\text{phosphate in 100 cc. of plasma}}$ analogous to the time relationship shown in table 3 for the corresponding urea ratios. In figure 5 these phosphate ratios have been plotted for each experiment against the time

TABLE 4

Effect of the ratio: $\frac{\text{rate of excretion}}{\text{plasma concentration}}$ *of the time elapsing between the injection of phosphate and the measurement of the ratio*

AVERAGE TIME FROM INJECTION TO MID-POINT OF PERIOD OF URINE COLLECTION	NUMBER OF OBSERVATIONS	RATIO: $\frac{\text{RATE OF EXCRETION}}{\text{PLASMA CONCENTRATION}}$	RATE OF EXCRETION—MGM. P PER KILO PER HOUR	PLASMA CONCENTRATION—MGM. P PER 100 CC.
minutes				
11	6	1.26	25.1	19.0
32	13	1.07	15.4	14.1
52	10	0.90	10.6	10.9
91	11	0.50	5.0	9.6
150	7	0.37	3.5	9.1
202	2	0.27	2.0	8.3

between injection and the midpoint of the periods of urine collection, and in table 4 the average ratios for the successive time intervals are tabulated.

Figure 5 and table 4 show that in general the closer the time at which the rate is measured to the moment of injection the greater is the magnitude of the phosphate ratio. That is to say, the kidney would excrete the phosphate at a greater rate soon after the injection than it would at any later time even though the plasma phosphate concentration remained the same. There is thus in the case of phosphate excretion a repetition in a more pronounced degree of the same phenomenon observed in the urea experiment we have cited. The only qualitative difference is the gradual acceleration of urea excretion after the oral administration of urea as contrasted with the immediate acceleration of phosphate excretion which follows the intravenous injection of phosphate. We know therefore that the change in the phosphate concentration of the plasma

which we produced was not the only variable in our experiments. There was also a continually varying degree of change in the balance between accelerating and inhibiting factors other than the concentration of phosphate in the plasma and this change in the value of a was just of such a nature as to produce the appearance of a phosphate threshold which is given in figure 3 or in other words to convert the formula $y = ax$ into $y = ax + b$.

If the time factor had been the only variable other than the plasma phosphate concentration the value of those ratios which were measured at the same time after injection would have been identical. But figure 5 indicates no such agreement so it would seem that still another factor was influencing the rate. Since the curves of the ratios of the various experiments tend to be parallel to one another, we may conclude that in the main this other factor is one of individual variation, or, more specifically, that the differences in the magnitude of ratios observed at any given time are conditioned by individual differences in the phosphate excreting capacity of the kidneys of different rabbits. These differences are not necessarily only of a physiological nature for it has been recently reported that structural changes may be produced in the kidneys of rabbits by the intravenous injection of acid or alkaline sodium phosphate (7) and we ourselves observed that albumen sometimes appeared in the urine after the injection of our neutral solution. Nevertheless by dealing only with ratios obtained from the same animal we can at least eliminate that part of the factor of individual variation which is dependent on constant physiological and structural differences. In some cases (see table 1) repeated experiments with varying amounts of phosphate were carried out on the same rabbit and if we restrict ourselves to a comparison of those ratio values which were measured in the same animal at the same time after injection we shall have a small number of more strictly comparable observations which may be of value in attempting to determine whether the rate is proportionately greater at certain levels of plasma phosphate concentrations than at others or may vary with changes in the concentration of plasma in the urine or with alterations in the volume of urine. These ratios are tabulated in table 5 along with the corresponding plasma and urine phosphate concentrations and urine volumes.

Although even these ratios show variations which must be attributed to uncontrolled variables they tend to be of the same order of magnitude in each animal and what is of particular importance for our problem such variations as occur show no constant quantitative relation to changes in the plasma phosphate concentration, urine phosphate concentration or urine volume. This fact in conjunction with the more general considerations we have outlined above lends support to the conception that the

rate of phosphate excretion varies as the plasma phosphate concentration and conforms to the formula $y = ax$. As a proof that this relationship exists our data are both qualitatively and quantitatively inadequate, but short of the amassing of such a bulk of figures as to admit of treatment by statistical methods it seemed to us that further work under the same necessarily inconstant conditions would be of little avail. So far

TABLE 5

Comparison of the ratios: $\frac{\text{rate of excretion}}{\text{plasma concentration}}$ *measured on the same animals at about the same intervals of time after phosphate injection but with variation in 1, plasma concentration, 2, urine concentration, 3, urine volume*

RABBIT	TIME FROM INJECTION TO MID-POINT OF PERIOD OF URINE COLLECTION	RATIO: $\frac{\text{RATE OF EXCRETION}}{\text{PLASMA CONCENTRATION}}$	PLASMA CONCENTRATION—MGM. P PER 100 CC.	URINE CONCENTRATION—MGM. P PER 100 CC.	URINE VOLUME—CC. PER KILO PER HOUR
	minutes				
5	44	1.23	15.4	283.5	6.7
5	35	1.02	13.2	160.7	8.4
5	42	1.15	9.6	72.8	15.1
5	93	0.55	9.1	52.1	9.6
5	104	0.57	7.1	27.2	15.1
9	35	1.90	13.9	213.0	12.4
9	41	1.13	13.9	296.2	5.3
9	37	1.20	7.7	105.7	8.7
10	36	1.52	13.8	337.1	6.2
10	35	0.83	10.7	86.4	10.3
10	96	0.48	9.3	67.2	6.7
10	94	0.28	8.0	27.9	7.9
11	40	1.41	13.2	229.7	8.1
11	35	1.13	11.8	166.3	8.0
11	97	0.44	9.1	54.8	7.3
11	95	0.43	7.6	27.7	11.9

as we can see the necessary degree of variation in plasma phosphate concentration can be achieved only by the intravenous injection of phosphate, a procedure which in itself introduces a new variable. Even when the effect of the time factor is as far as possible discounted there remains the factor of individual variation which cannot be eliminated simply by carrying out many experiments on the same animal because there is a danger that repeated injections of phosphate may introduce still another

variable dependent on the degree of structural damage done to the kidneys. There are thus special difficulties involved in the study of the effect of changes in plasma phosphate concentration on the rate of phosphate excretion, and it seems likely that a prerequisite for a completely satisfactory demonstration of the relationship is the discovery of more favorable experimental conditions than those which we have been able to devise.

Two papers which have appeared since our work was completed contain data obtained under other conditions than ours. Blatherwick, Bell and Hill (4) measured the rate of phosphate excretion in human subjects who had been given glucose and water. In half of experiments large doses of insulin were given, which tend to cause a decrease in the plasma phosphate concentration. They were not primarily concerned with the effect of changes in concentration on the rate, but it is of interest to note that any effect which the relatively slight alterations in concentration may have had is obscured by other factors under these particular conditions. Wigglesworth and Woodrow (8) carried out four experiments on themselves in which they measured rates just before and for five to six periods immediately after taking by mouth 25 grams of Na_2HPO_4 12 H_2O. This is a large dose of phosphate but the increase in the concentration of phosphate in the blood was very slight and the highest concentration attained was only 5 mgm. per 100 cc. Nevertheless they were able to show that the changes in the rate were roughly proportional to the changes in blood phosphate concentration. The plot of their rates against concentration inclined them to the view that there must be a phosphate threshold at or about 2.4 mgm. per 100 cc., but we do not believe that their threshold has any more actuality than our own.

In spite of all the deficiencies in the observations which are at present available, we believe there is enough evidence to warrant the hypothesis that if the conditions could be kept constant the rate of phosphate excretion would vary as the plasma phosphate concentration, in conformity with the equation $y = ax$. The experimental difficulties experienced in the direct verification of this hypothesis are dependent on the fact that a, which represents the resultant of all other variables than plasma phosphate concentration, does not commonly remain a constant, but is in a state of unstable equilibrium in accordance with shifts in the balance between those factors which accelerate and those which depress the rate.

CONCLUSION

By the intravenous injection of a neutral solution of sodium phosphate all variations in the phosphate concentration of the plasma consonant with freedom from tetany were produced while the rate of phosphate

excretion was being measured. When these observations are combined with others obtained under the same conditions except for the omission of the phosphate injection the data indicate that the rate per kilogram of body weight varies as 0.063 times the square of the plasma phosphate concentration. It is concluded, however, that this equation is only applicable to data obtained under these two diverse conditions. The measurements made after phosphate injection show more nearly a linear relationship of the form $y = ax + b$. But from a further analysis of the results it is concluded that the factor b, which indicates the existence of a renal threshold for phosphate, appears in the equation only as a result of a variation in the supposed constant a, and the conclusion is finally reached that the available evidence is in favor of the hypothesis that the rate varies as the plasma concentration and is expressed by the formula $y = ax$.

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ULTRA-VIOLET RADIATIONS IN CONDITIONS OF EXTREME CALCIUM AND PHOSPHORUS DEFICIENCY

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Received for publication December 27, 1924

Definite research for the causative factors in the nutritional disturbance known as rickets was begun by various observations as to its distribution and occurrence. In 1890 T. A. Palm (1) showed by a geographical survey that lack of sunlight seemed to be one of the chief factors in the causation of rickets. This led to the use of the sun bath in the treatment of rachitic children. In 1914 Rollier also mentioned rickets as amenable to sunlight therapy but did not discuss the possible rôle played by ultra-violet rays in calcium metabolism. Later the work of Finsen demonstrated that the portion of sun light spectrum of greatest therapeutic value lay in the ultra-violet zone. Hess (2) in 1918 demonstrated that the prevalence of rickets in the negro race could be accounted for in part at least by the factor of diet, as well as the rôle of fat-soluble vitamin and its relation to rickets. McCollum (3) and others demonstrated that rickets can be produced in animals if fed on a diet containing an excess of calcium but deficient in phorphorus and vice versa. It is now established by the work of many investigators that there are four factors: calcium, phosphorus, vitamin D and light rays, lack of any one of which plays an important part in the production of rickets. More recent studies of McCollum and Huldschinsky (4) have shown that ultra-violet rays from artificial sources are of value in the prophylaxis and treatment of rickets. It has been definitely shown that rickets can be prevented in animals if they are routinely exposed to ultra-violet radiation, while being fed on a diet unbalanced in calcium-phosphorus content, but otherwise adequate for growth.

Our present problem is to present additional evidence in confirmation of the previous findings, using a dietary somewhat more deficient in calcium and phosphorus than that used in previous experimental work. Former observations had demonstrated that the diet was below the optimum in phosphorus and calcium. Our problem was to determine if the radiations from the air-cooled mercury vapor lamp were sufficient to prevent rickets in rats fed solely on this deficient diet. A distance of 36 inches was selected for radiation, since the work of Hess and Weinstock (5) and other observers

has shown that ultra-violet rays of wave lengths from 2654 to 3022 Angström units are of greatest prophylactic value in rickets. The comparative findings in the three groups of each experiment were checked by daily observations, autopsy findings, x-ray studies and histological sections as in the previous work of Hess (5) and McCollum (6). In addition total chemical analyses were made to determine the calcium retention influence of the radiations given without filter at this distance, which eliminates rays shorter than those of proven value in this connection.

The rats in these experiments were divided into three groups: A, B and C. Those in group A, being on normal diet, were kept as controls. Groups

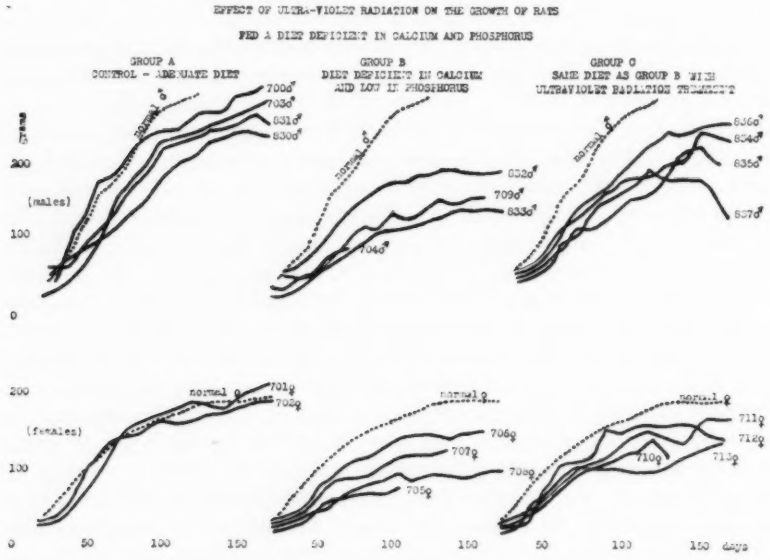

Fig. 1

B and C were placed on a rickets-producing diet; group B was kept in ordinary room light and C was given systematic ultra-violet radiations. When the rats were 170 days old, the experiments were terminated to permit a systematic study of the bone condition in all groups, as outlined in the previous paragraph.

The ultra-violet light exposures were given at a constant distance of 36 inches and the rats were so arranged in the cages that the dorsal half of the body was exposed to the rays. This 36 inch distance gave ultra-violet radiations of greatest intensity in the region of 3000 Angström units and eliminated the undesirable effect of the shorter chemical rays. The mini-

mum amount of conjunctivitis was due doubtless to the absence of the shorter and more irritating rays. Beginning with two minutes three times each week, the time was gradually increased by one and later two minute increments, until toward the termination of the experiment the total time of each exposure was 60 minutes in the first experiment and 50 minutes in the latter. It is assumed that the ultra-violet lamp had decreased in efficiency, as it had been also used for therapeutic purposes.

Selection of diet. Rats of the same age and approximately the same weight were selected for the experiments and the diets used were based on the Osborne and Mendel "synthetic" food with some modifications. The control diet contained slightly less than what McCollum (6) considers an optimum of both calcium and phosphorus but was entirely adequate for normal growth in group A. The contrast in growth between groups B and C on the rachitic diet is graphically illustrated in the weight chart.

Composition of diets

Control diet	*per cent*	*Low calcium and phoshorus diet*	*per cent*
Egg white	18	Egg white	18
Starch	42	Starch	42
Lactose	15	Lactose	15
Complete salt mixture	4	Calcium-free, low phosphorus salt mixture	4
Crisco	12	Crisco	12
Butter fat	9	Butter fat	9
0.4 gram yeast daily		0.4 gram yeast daily	

No vitamin D in the form of cod liver oil was furnished in either diet but a deficiency of this factor is of importance chiefly where the calcium or phosphorus is below optimum. The diets differed only in the composition of the salt mixture (7) used, but that was sufficient to reduce both calcium and phosphorus far below the minimum requirements for normal growth in the deficient diet. Distilled water only was furnished to all experimental animals. The general condition of the rats on the deficient diet was much poorer than the normal. The eight rats receiving the ultra-violet light, however, appeared brighter, more lively and had better fur than those on the same diet kept in ordinary room light. All rats on the experiment were kept in an ordinary room where the little sunlight which entered was filtered through glass, which as shown in the experiments by Hess and Weinstock (5) is sufficient to filter out all ultra-violet rays below 3022 Angström units. As an additional safeguard against interfering light factors, a dark cover was placed over some of the animals but this was omitted in the second group as no apparent benefit was gained from the room light.

Since the ultra-violet radiation seemed to benefit the animals in various ways, and x-ray photographs showed an increased calcification, it seemed advisable to determine the comparative calcium content of these animals. W. B. Lewis of the Chemistry Department made total calcium determinations by a modified Halverson and Bergheim (8) method on several of the rats from each group. The results are shown in the accompanying table correlated with the calcium and phosphorus content of the diet. It is evident that the ultra-violet light must have been responsible for the increased retention of calcium, since no other difference in diet or environment existed between the rats on deficient diet.

TABLE 1

Total calcium determinations on rats showing the effect of the calcium and phosphorus content of diet and ultraviolet radiation of rats on calcium retention

	RAT NUMBER	CALCIUM IN DIET	PHOSPHORUS IN DIET	WEIGHT OF RAT		PERCENTAGE CALCIUM	
				Moist	Dry	Moist	Dry
I.	700	0.561	0.386	284.7	101.5	0.978	2.73
	701	0.561	0.386	198.0	76.3	1.31	3.395
	702	0.561	0.386	175.0	66.1	1.29	3.29
	703	0.561	0.386	262.0	91.1	0.92	2.62
II.	706	0.021	0.206	141.2	50.5(?)	0.717	2.0
	708	0.021	0.206	93.4	31.4	0.763	2.27
	709	0.021	0.206	147.0	53.1	0.693	1.78
III.	711	0.021	0.206	153.5	50.9	0.912	2.74
	713	0.021	0.206	116.0	43.4	0.847	2.26
Group I. Adequate diet—Average total calcium.....						1.124	3.009
Group II. Low Ca and P—Average total calcium.....						0.724	2.01
Group III. Low Ca and P with ultraviolet treatment—Average total calcium.....						0.879	2.5

Careful autopsy examinations were made on all rats not used for total calcium determinations. Those on the control diets were practically normal. Those on the deficient diet kept in an ordinary room showed definitely beaded ribs, curvature of spine, stunted growth and enlarged spleen. Rats on the deficient diet receiving ultra-violet treatment showed no beading in three cases and very slight on the right side of the fourth. Two of group C, that died before the completion of the experiment, seemed to have suffered from an internal hemorrhage. Enlarged spleen was a rather constant finding in all the animals on this experiment. Further research is being conducted in an attempt to correlate the variations in the size of the spleen with changes in dietary factors.

The roentgenographic studies of skeletal structures of the animals on

this experiment were made in two stages for comparative observation of bony structure, special attention being given to the epiphyseal ends and shafts of the long bones. The first x-ray studies were made during the fourth week of the experiment. Even at this early period we found rather marked difference in size (total skeletal structure) and changes in epiphyseal areas. There was uniformly a retarded skeletal development in group B. Spinal curvature and rib changes were present and there was appreciable epiphyseal enlargement. The total enlargement may not be visualized because of a lack of calcium deposit. These changes were so minimal in group C that they could easily have been confused with the normal or control group except for the epiphyseal enlargements of the tibia and femur.

The second roentgenographic studies were made at the time of terminating the experiment. In these films the comparative findings were much more evident. In the rachitic group B, the skeletal development was markedly below par. Spinal curvatures, beaded ribs and epiphyseal enlargements were uniformly present. In the long bones of this group there were areas of rarefaction and the porosity at the epiphysis gave the bony structure a spongy appearance. In group C the cortex of the bone shaft is well formed and symmetrical. The epiphysis while somewhat enlarged had a definitely formed line separating the shaft and the cartilage, as shown in the photographs.

The metaphyseal line was irregular and ill defined in group B and there was a much wider intervening zone between shaft and cartilage. The more definite line in group C was characteristic of healing rickets and compared favorably with the clean-cut line seen in the same zone in the control group A.

The characteristic abdominal distention occurring in all animals on rachitic diet was observed. X-ray study showed it to be due to a rather uniform distention of the gastro-intestinal tract. We were unable to observe any evidence of beneficial effect of light therapy in relieving this condition in these animals.

Histology. The sections chosen for histologic study were taken from the proximal end of the tibia and the distal end of the femur. Representative sections were made from the three groups. In group B the sections showed a very thin spongy bone cortex, as compared with the normals in A. The bone trabeculae were small and less numerous while the intervening spaces were larger than in the other two groups and the transition zone much broader. There was a minimum amount of calcification in all sections studied in B, while in C the process of calcification (bone formation) approached so near the normal as to be hardly distinguished from the sections in group A, except that cartilage cells were slightly more numerous in the bone trabeculae, giving the appearance of a widened transition. There

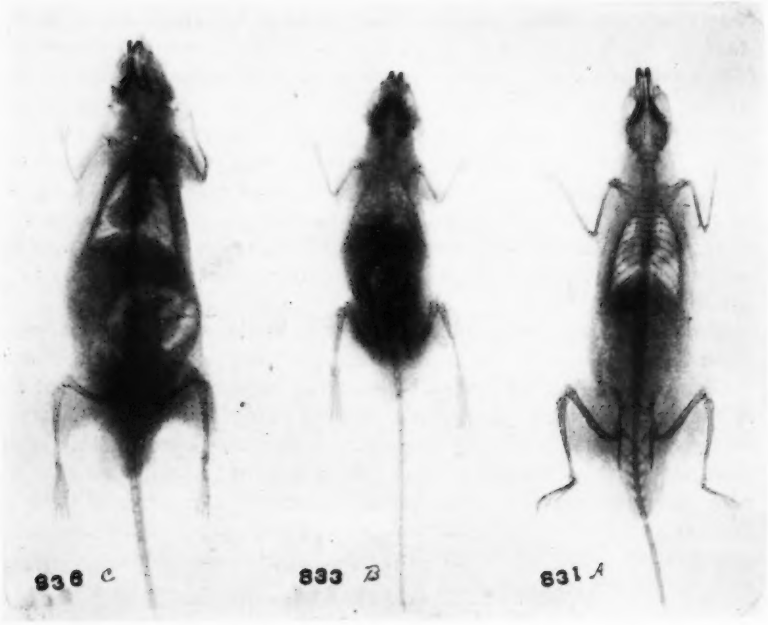

Fig. 2. Rat 831. Normal Diet. No. 833. Rat on rachitic diet. No ultraviolet radiation. No. 836. Rat on same diet and conditions as no. 833 except given ultraviolet radiations.

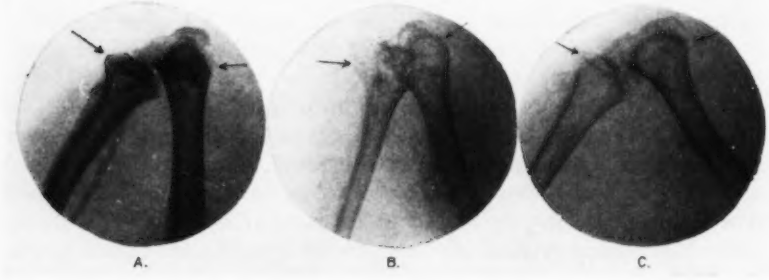

Fig. 3. A. Normal bone with well defined metaphyseal line.

B. Enlarged epiphysis in rachitic rat; corresponding joint as in A. Note irregular line of ossification (arrow).

C. Corresponding joint from rat on rachitic diet given ultra-violet radiation.

was a sharp demarcation zone between the shaft and cartilage in group C, as well as in group A, as shown by the microphotographs.

Blood vessels and fibrous tissue are more numerous in the epiphyseal area in group B. Transverse sections in group B are wider than in either

Fig. 4. A. Normal bone showing narrow metaphyseal zone as compared to that seen in B.

B. Microphotograph of section of metaphyseal zone in rachitic animal. Note broad transition zone.

C. Microphotograph of metaphyseal zone of tibia in rat on rachitic diet, given ultra-violet radiation. "Line test" of McCollum comparable to that of normal bone in A.

of the other two groups, giving the typical appearance of rachitic bone. In sections of group C, the osteogenic spicules were as numerous and presented apparently the same amount of calcium deposit as in normal sections. From microscopic study the sections in group B were typical of bone formation in severe rickets. Group C could be classed as well

healed early rickets. It is evident that the ultra-violet radiations in this experiment were sufficient to prevent the continued development of rachitic bone processes in all sections studied. The so-called "line test" of McCollum (9) seems to be well defined in these sections.

SUMMARY

1. A diet deficient in calcium, phosphorus and vitamin D was used to produce a rachitic condition in rats while the control diet contained optimum amounts of both calcium and phosphorus.

2. The ultra-violet light exposures were given at constant distance, 36 inches, the time factor being increased as rapidly as previous experience and the condition of the animals indicated advisable.

3. Daily observations of growth and general condition showed group A normal; group B stunted and deformed; and group C closely approaching the normal.

4. Total calcium determinations showed an average of 3.009 per cent of calcium in group A; 2.01 per cent in group B; and 2.5 per cent in group C. This and the general findings indicate at least a 50 per cent improvement as a result of the radiation.

5. Autopsy examinations showed definite rachitic condition in group B with marked improvement in group C.

6. Roentgenographic studies made early and at the close of the experiment showed normal bone in group A, definite rachitic changes in the bones of group B, and evidence of healing rickets in group C.

7. Histological studies confirmed the so-called "line test" of McCollum, showing the healing effect of the ultra-violet radiation.

8. Ultra-violet light not only aids in healing of rickets caused by deficiency of either phosphorus or calcium, as shown by other workers, but causes a retention of calcium when both factors are deficient and a consequent beneficial effect on the skeletal development and the organism as a whole.

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AN ANALYSIS OF CERTAIN MECHANICAL CONDITIONS IN THE PULMONARY CIRCULATION AND THEIR EFFECTS UPON LUNG VENTILATION

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Received for publication January 13, 1925

In 1921, Drinker, Peabody and Blumgart (1) reported a group of experiments designed to test the extent to which pulmonary congestion could diminish lung ventilation. These experiments made use of a new heart preparation (2) in which the lungs were enclosed within the pleural sac and the heart and great vessels exposed for manipulation. The cats utilized were anesthetized with urethane and curarized. They were given artificial respiration by means of a simple apparatus which delivered air with a degree of constancy quite great enough for the experiments in question. The principle upon which this apparatus was constructed is shown in figure 1. Air is driven into *A* and passes through tracheal cannula *D* to the lungs, *E*. During the positive blast of the pump, cut-off hammers *1* and *2* are open and air may enter the lungs or, in part, by-pass through *B* for collection in a delicately balanced spirometer—the overflow spirometer. Expiration depends upon the passive collapse of chest and lungs and delivers air out through *C*, hammers *1* and *2* being down during this event and hammer *3* being up. All of the expired air may be collected in a second similar spirometer called the expiration spirometer. It is clear that if the apparatus driving air into *A* does so in constant volume per minute, the amounts of air collected in the overflow and expiration spirometers in given periods of time will add together to the same total, and this will be true no matter how widely the ratio between the two volumes may vary. If anything occurs which hinders the entrance of air to the lungs, the overflow spirometer collection will rise and the expiration spirometer volume will fall by a similar amount. The ratio between the two volumes thus becomes an index of air capacity in the lungs.

In brief, these early experiments showed that, when the pulmonary veins were compressed and the lung capillaries thus stuffed with blood, the amount of air reaching the alveoli was reduced markedly. The authors concluded that this result had a definite explanatory bearing upon the reduction in vital capacity which accompanies cardiac decompensation

in certain cases of valvular heart disease. But even in terms of the simplest mechanical considerations the experiments left many factors unmeasured. For example, the opinion was expressed, in the paper referred to (1), that the changes in ventilation which were described were not accompanied by serious alterations in pulmonary arterial pressure. This statement did not rest upon satisfactory experiment, since at the time it was impossible to record this pressure and retain a reasonable degree of circulatory integrity in the animal. The present paper deals with observations in which the same animal preparation has been employed and in principle the same arrangement of respiratory apparatus. By the use of a simple trocar cannula (3) it has been easy to obtain pressure readings in the pulmonary artery during periods of pulmonary congestion and altered ventilation. It has thus been possible to test what was formerly opinion, and to gather certain correlated facts which seem to have bearing upon the general problem of cardiac decompensation.

EXPERIMENTAL. *A. The animal preparation.* The cats employed in these experiments have been anesthetized with urethane (2 grams per kilo by stomach tube), or else with barbital sodium (0.3 to 0.6 gram per kilo intraperitoneally), and have been operated upon in the manner described in previous papers (1), (2). Further experience has shown that it is possible to close the chest by means of the pericardium so as to render it absolutely tight in about three out of five attempts. When small leaks occur they are extremely hard to find, but their existence cannot escape notice. The lungs are readily seen through the transparent pericardium and should fill all the available space in the chest. If they begin to fall away from the under surface of the pericardium, a leak exists and the operation is at fault. Furthermore, when a small leak is present, the lungs slowly collapse and quite a large air pressure is necessary in order to inflate them, owing to the fact that the air imprisoned in the thorax must be driven out through the minute opening constituting the leak at each positive blast of the artificial respiration. Since the air inflow tube is connected with an overflow device, animals with leaky chests suffer rapidly. The air designed to inflate their lungs meets such a degree of obstruction that most of it is shunted out the overflow tube. Under such circumstances the heart becomes cyanotic, dilated and feeble, and the systemic blood pressure falls. A compelling type of control is thus imposed, since it becomes futile to attempt experimentation with animals unsuccessfully prepared.

The ligature and clamp used in compressing the pulmonary veins are essentially unchanged from our previous description (1). In placing the ligature around these veins it is safer to dissect the path used rather than to thrust a ligature carrier through the tissue surrounding the vessels. If it is desired it is easy to place this ligature when the pericardium is

first opened and before sewing the margins of the pericardial slit to the chest wall. When this is done at this early stage in the operation, it is easy to see whether the dissection used in carrying the ligature around the pulmonary veins has pierced the pleura, thus spoiling the closed chest character of the experiment. A little experience will, however, demonstrate that this accident is not frequent, and the operator will prefer to place the ligature after the pericardium has been sewn into final position.

B. The normal pressure in the pulmonary artery of the cat. During the past two years we have made over three hundred measurements of pulmonary arterial pressure in anesthetized cats. In some cases the chest has been closed and the animal breathing naturally. In others the chest has been open and artificial respiration has been used. In still others the chest has been closed and the artificial respiration employed following

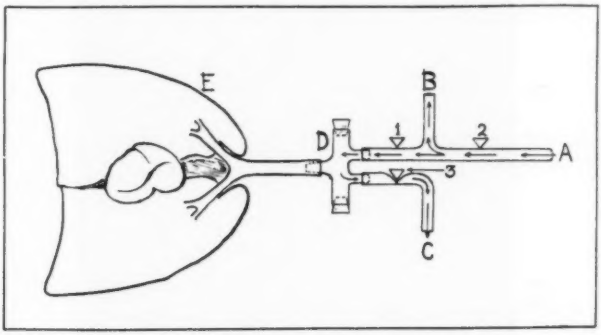

Fig. 1. Diagram illustrating the principle applied in artificial respiration. *A*, air inflow; *B*, overflow tube leading to overflow spirometer; *C*, air outflow leading to expiration spirometer; *1* and *2*, cut-off hammers to occlude inflow tube during expiration; *3*, cut-off hammer occluding outflow tube during the inspiratory blast of the pump; *D*, tracheal cannula; *E*, trachea and lungs.

curarization. We have been impressed by the fact that the initial pressures obtained in different animals vary quite markedly. A glance at table 1 will illustrate this, and will show that low pulmonary pressures do not of necessity correlate with low systemic pressures. Thus, in animal 7 there is a systemic pressure of 104 mm. of mercury and a pulmonic pressure of 14, and in no. 14 a sytemic pressure of 84 is accompanied by a pulmonic pressure of 9. Variations in this direction seem to depend more upon rate of venous inflow into the right side of the heart than upon any other factor—a point which can be shown quite diagrammatically by means of the modified Starling heart preparation now in use in this laboratory. Tracing A in figure 2 is the pressure in the pulmonary artery in such a preparation, and C is the systemic blood pressure. At the mark, *E*, the venous inflow is shut down from 150 cc. per minute to 26 cc. per minute.

This results in a prompt fall of pulmonary arterial pressure from 23 mm. of mercury to 13 mm. of mercury. Owing to the fact that the preparation possesses a perfectly competent constrictor apparatus, the systemic pressure does not fall at all.

In the light of such an experiment we are inclined to believe that animals

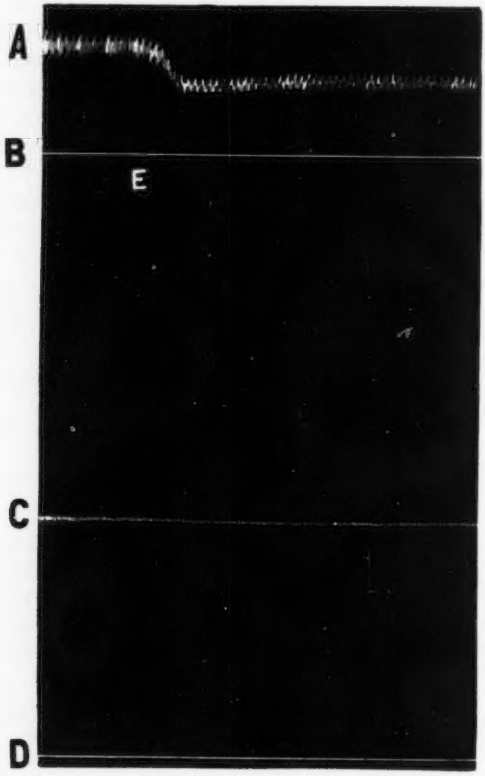

Fig. 2. *A*, pressure in the pulmonary artery written by means of a membrane manometer; *B*, base line for this pressure; *C*, systemic blood pressure; *D*, base line for systemic pressure. At mark *E*, the inflow to the right side of the heart was reduced from 150 cc. to 26 cc. per minute.

such as no. 7 and no. 14 represent cases in which the vasomotor system is highly competent and is maintaining a good head of pressure in the carotid artery. But the actual volume of blood in motion on the arterial side is greatly reduced and the venous inflow into the right side of the heart comparatively small. The pulmonary vascular bed being

TABLE 1

Pressures in the femoral and pulmonary arteries of 36 cats before and after a wide variety of experimental procedures

NUMBER	WEIGHT	MEAN PRESSURE		CHARACTER OF EXPERIMENT	MEAN PRESSURE	
		Femoral artery	Pulmonary artery		Femoral artery	Pulmonary artery
	kgm.	*mm. Hg*	*mm. Hg*		*mm. Hg*	*mm. Hg*
*1	3.6	140	30	3 compressions pulmonary veins (2 gear shifts)	125	27
*2	3.2	139	28	2 compressions pulmonary veins (1 gear shift)	130	31
*3	3.5	120	31	Prolonged compression pulmonary veins	36	31
*4	3.5	118	22	2 compressions pulmonary veins (2 gear shifts)	18	7
5	4.3	116	19	2 compressions pulmonary veins. Compression superior vena cava	96	20
*6	3.6	104	22	3 intravenous injections adrenin	82	23
*7	3.9	104	14	3 compressions pulmonary veins (2 gear shifts)	60	12
*8	3.5	100	27	Prolonged compression pulmonary veins	90	24
9	4.0	94	23	Pulmonary veins and superior vena cava compression	72	18
*10	3.5	90	26	Prolonged and extreme compression pulmonary veins	80	24
*11	3.2	90	14	3 compressions pulmonary veins	132	22
*12	4.4	88	21	Compression pulmonary veins	50	20
*13	3.6	86	32	2 prolonged compressions pulmonary veins	26	29
*14	2.7	84	9	2 compressions pulmonary veins	50	7
15	3.2	84	20	2 compressions pulmonary veins. Compression superior vena cava	52	14
*16	3.1	80	20	2 compressions pulmonary veins	38	17
17	3.2	76	22	3 compressions pulmonary veins	74	21
18	3.0	76	8.5	3 compressions pulmonary veins. Vagi cut	38	5
*19	3.7	73	22	2 compressions pulmonary veins	42	20
*20	3.8	73	19	Prolonged compression pulmonary veins	24	17
*21	3.0	70	23	Compression pulmonary veins. Intravenous adrenin. Over-ventilation	20	14
*22	2.6	64	11	Compression pulmonary veins. Intravenous 15 per cent NaCl	21	9
23	2.5	64	12	Compression pulmonary veins. Vagi cut	49	10
*24	2.8	63	20	Intravenous adrenin and later 15 per cent NaCl	60	23
*25	2.8	62	20	Prolonged compression pulmonary veins twice	20	11
*26	3.5	60	24	Compression pulmonary veins	40	21
*27	3.1	60	16	Compression pulmonary veins twice	30	14
*28	3.9	54	28	Compression pulmonary veins twice	64	28

TABLE 1—*Concluded*

NUMBER	WEIGHT	MEAN PRESSURE		CHARACTER OF EXPERIMENT	MEAN PRESSURE	
		Femoral artery	Pulmonary artery		Femoral artery	Pulmonary artery
	kgm.	*mm. Hg*	*mm. Hg*		*mm. Hg*	*mm. Hg*
*29	2.4	54	17	Prolonged compression pulmonary veins. Intravenous adrenin and 15 per cent NaCl	31	14
30	3.6	52	10	Inhalation 10 per cent CO_2	45	7
*31	3.2	50	12	Compression pulmonary veins twice. Intravenous adrenin and 15 per cent NaCl	18	8
*32	3.9	50	27	2 compressions pulmonary veins	64	32
*33	2.7	46	23	Intravenous adrenin 3 times	36	23
*34	2.9	45	20	Prolonged compression pulmonary veins	22	13
*35	2.4	44	14	Compression pulmonary veins twice. Intravenous adrenin	30	13
*36	3.0	40	13	Compression pulmonary veins twice	10	10
Av.	3.3	78.1	19.7		52.1	17.8

* Indicates artificial respiration with apparatus described in text; all others, natural breathing.

enormous and exceptionally elastic, the result is a low pressure in this circuit. For the same anatomical reasons, coupled with absence of vasomotor control of significant grade and apparently also with absence of capillary contractility, the pulmonary arterial pressure in any animal resists change under the ordinary conditions of laboratory experiment. Given a competent heart muscle, cardiac inflow is apparently the greatest controlling factor, and this changes but slowly in the quiescent anesthetized animal. Under conditions of hard muscular effort we believe the pulmonary pressure must increase greatly. The right ventricle is surprisingly able to meet obstructions appropriately placed in the pulmonary circuit. In our hands attempts to obtain a sustained increase in pulmonary arterial pressure by gradual compression of the pulmonary artery have ended in failure. The lumen of the artery may be narrowed to a certain point without materially changing the pressure. Then, suddenly, the pressure rises momentarily but falls as the right ventricle dilates and fails. Apparently, when the right ventricle is confronted by a relatively inelastic obstruction such as exists in this experiment, it fails with extreme promptitude. In order to see prolonged and large rises in pressure in the pulmonary artery it is necessary that the obstruction be far out in the circuit, either in the neighborhood of the capillaries or beyond them. Obstructions so placed exert their effects gradually, owing to the vast size and elasticity of the pulmonary circuit. Under these circumstances the right ventricle meets the strain with surprising success.

Buetner (4) has published measurements of pulmonary arterial pressure in seven cats. He used an open chest preparation and cannulated a large branch of the pulmonary artery, thereby cutting off a considerable section of the pulmonary arterial bed. He employed a mercury manometer and the average mean pressure for the seven cases was 17.6 mm. of mercury with a variation between 24.7 and 12.8 mm. Our measurements have employed the pulmonary arterial cannula heretofore described (3), together with a membrane manometer, and have been made with the

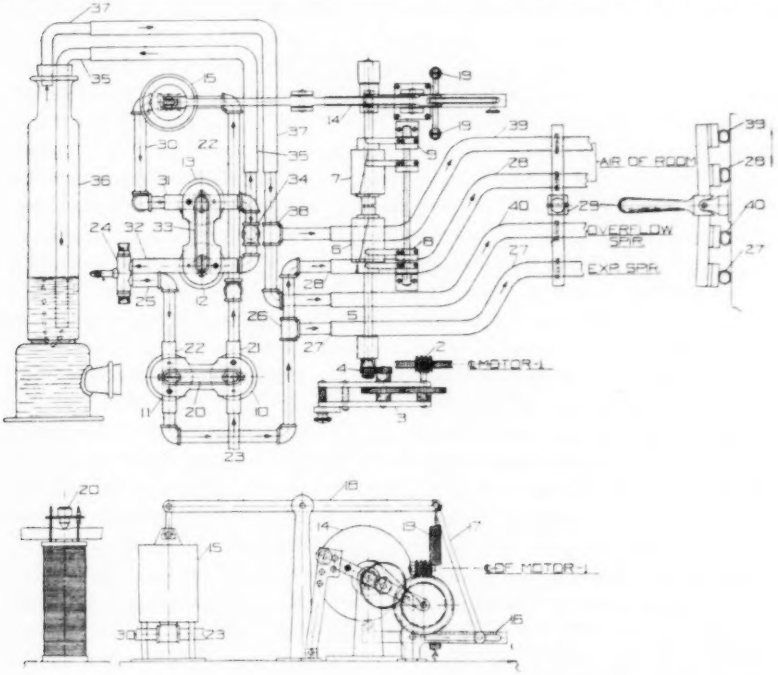

Fig. 3. Respiration pump. Air delivery constant under conditions of constant pressure.

pulmonary bed unrestricted. In table 1, a series of figures is presented covering experiments selected more or less at random from a large group of observations. The average for these measurements taken prior to different radical manoeuvres is 19.7 mm. of mercury, and it will be seen that the pulmonary pressure does not display a fall proportionate to the systemic.

C. The apparatus for artificial respiration. Figure 3 represents a respiration pump and valve mechanism which will deliver remarkably

constant volumes of air. This pump supplants the water pump arrangement used in former experiments, and possesses flexibility and reliability in high degree.

The apparatus consists of a one-quarter horse power motor *1* not shown in the figure, the shaft of which carries a worm gear *2* which in its turn engages a movable gear train *3*. This train engages gear *4* which is pinned to shaft *5*. Variations in the speed of the pump are secured by shifting gears either in the train *3* or at *4*. Eight such changes are possible and the speed of the motor is constant for any setting of a centrifugal contact-breaking speed regulator. The shaft *5* carries two combination brass and vulcanite cylinders *6* and *7* upon which rest two sets of brushes *8* and *9*. Cylinder *7* and brushes *9* make and break an electric circuit which activates two pairs of electromagnets *10* and *11*. Cylinder *6* and brushes *8* make and break a similar circuit activating magnets *12* and *13*. On the end of shaft *5* there is a cam *14* which rotates with the circuit-breaking cylinders *6* and *7*, and drives the pump *15* through the lever arrangement *16*, *17*, and *18*. As the cam *14* rotates it plays upon lever *16*, being kept in contact with it by two strong springs *19*. This motion is imparted to the walking beam *18* through lever *17* which may be set at various points on the scale on *16*, thereby giving the desired stroke or thrust of the pump.

If now the movement of air through the apparatus is followed the general method of operation will be clearer. Consider that the piston of the pump *15* is at the bottom of the stroke. As it begins to rise the circuit leading to electromagnets *10* and *11* is broken. The soft iron cut-off *20* is released and the elasticity of the rubber tubes *21* and *22* causes them to open. Air or any desired gas mixture is drawn into the pump through tube *23* in the direction of the arrows. During this filling phase of the pump which lasts one-half of a respiratory cycle, rubber tube *22* is also open and the animal exhales in a natural manner through the tracheal cannula *24*, via tube *25*. When this expired air reaches union *26*, two destinations are possible, *27* or *28*. The lever arm *29*, if thrown to the left, compresses two pairs of rubber tubes *27* and *40*. If thrown to the right two other tubes *28* and *39* are compressed. By this means the expired and also the overflow air can be turned out into the air of the room or can be collected in delicately balanced oil-floated spirometers. If, now, we consider that the piston of the pump *15* has reached the top of the stroke and is about to descend, the magnets *10* and *11* are reactivated and draw down the cut-off *20*, thus shutting off tubes *21* and *22* and making it necessary that the downward thrust of the pump drive air toward the tracheal cannula through tube *30* and rubber tubes *31* and *32*. These rubber tubes have sprung open as the down stroke of the pump begins, owing to release of the iron cut-off *33* as the magnetising circuit is broken

in *12* and *13*. The animal receives a gentle blast of air during one-half of the respiratory cycle, at the end of which magnets *12* and *13*, being reactivated, draw down the cut-off *33*, thus closing the blowing side of the device and leaving all in readiness for the initiation of the suction phase. The air driven toward the tracheal cannula is regulated as regards pressure by an overflow arrangement. A T-tube *34* permits the air to pass through *35* to a water-bottle, *36*, from which it may escape via tube *37* if the height of the water column is less than the pressure needed to achieve pulmonary inflation. The overflow air leaves the pressure regulating bottle by tube *37* and at union *38* two possible paths are provided, the first *39* leading to the room air, and the second *40* to the overflow spirometer. Lever *29* thus shunts all the overflow and all the expired air into the collecting spirometers or out to the room.

The pump is adjustable as to rate and thrust, and owing to the non-leakable magnetic compression valves delivers required volumes of air with a high degree of accuracy. Inspiration is a gentle blast. Expiration is in the natural manner without suction.

D. Experiments. It is unnecessary to give complete protocols of experiments since the entire list of procedures employed is shown graphically in figures 4, 5, 6, 7, 8 and 9. Explanatory notes will suffice for each case.

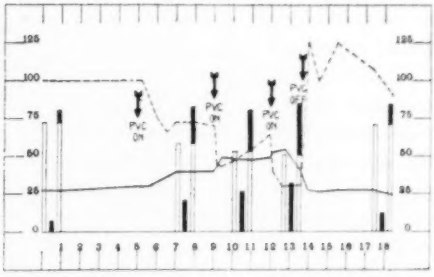

Fig. 4. Experiment 1. Broken line, systemic blood pressure; solid line, pulmonary arterial pressure; open columns, centimeters of air collected in expiration spirometer per minute; solid columns, air collected in overflow spirometer per minute; combined columns, total volume of air moved per minute. Reading from left to right the first three arrows indicate the moments when the pulmonary veins were compressed in succeeding steps. At the fourth arrow the pulmonary vein clamp was entirely released. Ordinates, millimeters of mercury; and, multiplied by 10, centimeters of air. Abscissa, time in minutes. Rate of artificial respiration 62 per minute. Maximum pressure of tracheal air in each blast 3 cm. of water.

1. Figure 4, experiment 1. Tightening of the pulmonary vein clamp began at the first arrow after 5 minutes of observation, during which the first air collection was made. Systemic pressure falls almost at once, indicating the fact that blood which should enter the left side of the heart is being stored in the lungs. Pressure in the pulmonary artery soon rises, but it is evident that the extremely distensible pulmonary bed receives a certain amount of blood before the obstruction begins to register its effects as pressure increases. The amount of pulmonary vein compres-

sion employed increases the pressure in the pulmonary artery from 30 mm. of mercury to 40 mm. of mercury, and this change is accompanied by a shift in air distribution in the direction of exclusion of air from the lungs and collection in the overflow spirometer. Observe that the systemic blood pressure reaches a low level 1½ minutes after the pulmonary vein compression becomes effective and then rises slightly. This tendency toward recovery is even more marked after the second and further tightening of the pulmonary vein clamp, and is seen to an extraordinary degree in figure 9. When blood is first prevented from reaching the left side of the heart but still runs in fair volume into the right side for storage in the pulmonary circuit, there is, first of all, an acute drop in systemic blood pressure—a pure expression of inadequate left ventricular filling. If, however, the animal is in good condition he compensates for this situation with an arterial vasoconstriction. Furthermore, if the right ventricular muscle is adequate and the pulmonary vein obstruction not too extreme, the enlarging pulmonary lake is soon filled and the driving pressure of the right ventricle may then greatly improve the filling of the left, so that the animal has more blood to feed into a constricted periphery and may show practically a complete recovery of systemic blood pressure in the presence of a greatly increased pulmonary arterial pressure.

Note the rise in pulmonary arterial pressure following the second and further tightening of the pulmonary vein clamp and the further decrease in the air actually reaching the lungs. After the third air collection, the clamp is given a last turn and the pulmonary veins reach a final stage in their progressive occlusion. Systemic blood pressure falls sharply to 30 mm. of mercury, and pulmonary arterial pressure, after reaching a height of 55 mm., falls until the compressing clamp is released, when, accompanying the rise in systemic blood pressure, it drops to the level noticed at the beginning of the experiment. There is a further slight decrease in the amount of air entering the lungs during this final period of compression. The situation presented by the animal in the last period is an interesting one and deserves comment. Figure 5 shows sections of the actual tracing in this experiment. As a result of the last clamp tightening, pulmonary arterial pressure rises and then falls, and the animal would go on to death if clamp release did not provide relief. The situation seems to have its explanation on the nutrition of the right ventricle. Coronary flow, in this case, depends on the balance between aortic pressure and pressure within the right auricle. If aortic pressure, through poor filling of the left side of the heart, becomes so low as to produce a coronary blood flow unequal to caring for the right ventricular muscle, then this muscle fails to maintain the abnormal pressure head in the pulmonary circuit. Figure 6, from another animal, illustrates the same set of phenomena but distributed in a more diagrammatic manner. In this case

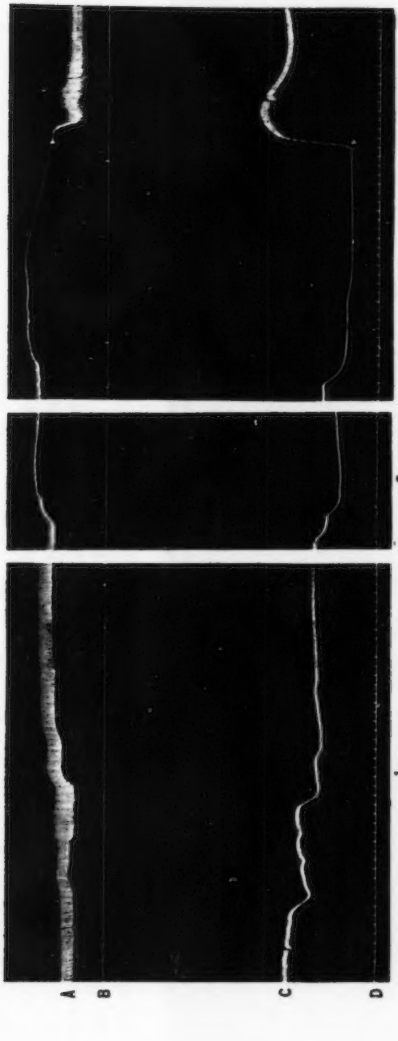

Fig. 5. Tracing of experiment 1. *A*, pulmonary arterial pressure, membrane manometer; *B*, base line for this pressure; *C*, systemic blood pressure, mercury manometer; *D*, base line for this pressure. *1*, *2* and *3* represent sections of tracing during the three progressive closures of the pulmonary vein clamp and the period of complete clamp release.

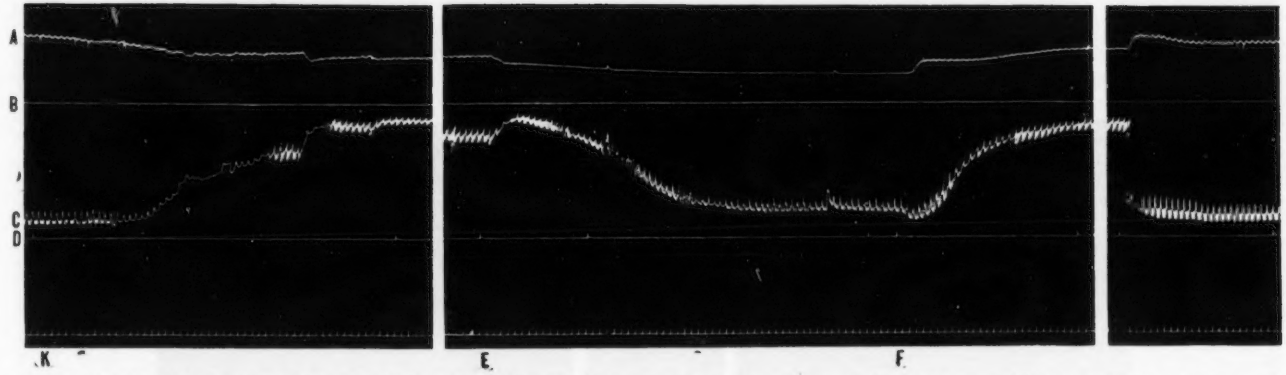

Fig. 6. Tracing showing the effects of reducing inflow into the left ventricle on the ability of the right ventricle to maintain pressure. *A*, systemic blood pressure, mercury manometer; *B*, base line for this pressure; *C*, pulmonary arterial pressure, membrane manometer; *D*, base line for this pressure. Section 1 shows the first tightening of the compression clamp for the pulmonary veins. Section 2, the further tightening of the compression clamp at *E* with resulting eventual fall in pulmonary arterial pressure, and at *F*, release of the clamp to the compression value present in section 1 and at the very beginning of section 2. Section 3 shows complete release of pulmonary vein compression with fall of pulmonary arterial pressure to normal.

pulmonary vein compression is applied first at K. When, as a result of too great pulmonary vein compression, the additional pressure being applied at E, the right ventricle fails, as between letters E and F of section 2 in the tracing, the animal enters a vicious circle with death as the only possible interruption. The detailed course of events in the experiment shown in figure 6 is given in the legend accompanying the illustration.

Returning to figure 4, it will be seen that with release of the compressing clamp systemic blood pressure shoots up above the original normal level as the volume of blood stored in the lungs rushes over into the systemic circuit. Pulmonary arterial pressure falls to normal and the final air collection shows practically a return to the original values, a situation which does not obtain if vein compression has induced any degree of pulmonary edema.

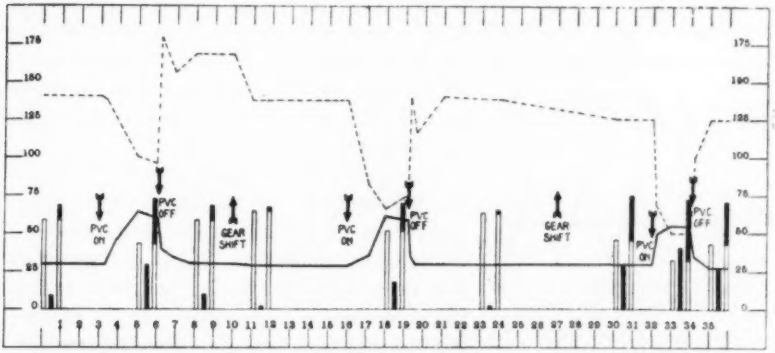

Fig. 7. Experiment 2. General arrangement of chart identical with figure 4. Three separate but equally extensive periods of pulmonary vein compression have been employed. Term *gear shift* explained in text.

This experiment shows quite conclusively that interference with air inflow into the lungs not due to edema but due to intravascular blood is accompanied by very substantial increases in pulmonary arterial pressure. It has been possible only once, and that in the experiment shown in figure 6, to obtain an air collection during such a period as is found between E and F, when blood is still stored in the lungs but is held there under very little pressure. In this particular case there was a very slight increase in the volume of air actually reaching the lungs during this period as compared with the preceding collection, when pulmonary arterial pressure was high. The difference is, however, too slight to deserve much comment.

2. Figure 7, experiment 2. In this experiment the character of the artificial respiration has been altered so as to contrast periods in which air was driven into the lungs at different pressures. In experiment 1, the respiration pump was set so as to deliver a trifle over 800 cc. of air per

minute at a rate of 62. Owing to the setting of the overflow bottle, *36*, figure 3, the maximum air pressure which could be reached in any single blast was 3 cm. of water. Under this setting, pulmonary vein compression, with resultant congestion of the lungs, brought about a diminution in the amount of air which entered the lungs. Owing to the low air pressure employed, which must be diminished to an extreme degree in the alveoli. the opportunity for intravascular congestion to interfere with air flow is maximal in such an experiment as this. It now became desirable to see how far pulmonary congestion could exclude air from the lungs, if what we may call the inspiratory blast was delivered under conditions providing greater pressure. The adjustability of the respiration pump makes it possible to do this under the circumstances indicated diagrammatically in figure 8. In this case a pump delivers, let us say, a total of 100 cc. of air per minute in eight blasts—line *A*. The stroke of the pump is now increased and the rate diminished so that 100 cc. per minute are delivered in four blasts—line *B*. If, in both cases, the air is delivered into an elastic bag, the maximal pressures reached in any blast will differ in the two cases, being far less in *A* than in *B*.

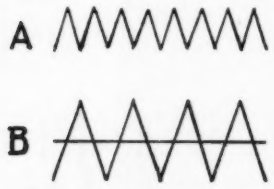

Fig. 8. Diagrammatic representation of the conditions of artificial respiration employed in experiment 2, figure 7.

In experiment 2, figure 7, this contrast of delivery of approximately the same quantities of air but under conditions of differing maximal pressures is exactly what has been accomplished. At the start of the experiment the pump rate is 63 per minute and the maximum pressure possible, arranged by adjustment of the water level in overflow bottle *36*, figure 3, is 3.3 cm. of water. A period of air collection is followed by the first compression of the pulmonary veins. The clamp used to provide compression carries a scale, permitting identity of adjustment in successive compressions. It is tightened to mark 15 in this and in the two following compressions. The result in the first case is a fall in systemic blood pressure (broken line), a rise in pulmonary arterial pressure (solid line), and a decided diminution in the amount of air actually reaching the lungs. When pulmonary vein compression is removed there is a return to normal, except that systemic blood pressure remains elevated for a considerable period.

At the first arrow marked *gear shift*, the rate of the respiration pump is reduced to 24 per minute. In order to move the same total amount of air per minute as in the first three collections, it is now necessary to increase the stroke of the pump so that more air is delivered at each blast, and in order to get comparable amounts of this air into the lungs the level of the water in the overflow bottle must be raised to 7.4 cm. This means

that in air collections 4, 5 and 6, the maximum pressure reached in the artificial respiration system is 7.4 cm.—more than double the value in the first three collections. This height was a trifle too great since it did not permit exactly the same relation between expiration and overflow air collections as seen in collections 1, 2 and 3. The error of adjustment, however, is in the direction of too great pressure in the inflow line and this is desirable rather than harmful for the experiment.

Under these conditions of artificial respiration, air collection 4 was made, and shortly after the pulmonary vein clamp was again tightened to exactly the same degree as in the first period of compression. There is again a shift in air distribution seen in collection 5, taken during the period of pulmonary vein compression, so that even with the increased inflow pressure the pulmonary congestion produced interfered with air entrance.

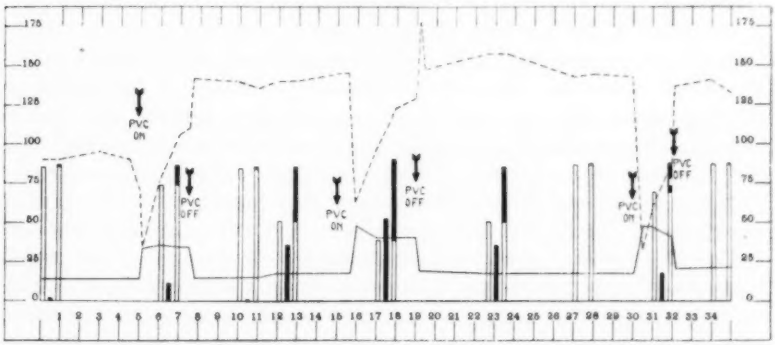

Fig. 9. Experiment 3. General arrangement of chart identical with figures 4 and 7. Experimental procedures described in text.

After releasing the pulmonary vein clamp, conditions return at once to normal as seen in the two blood pressure curves and in air collection 6.

For the final phase of the experiment the respiration pump rate was was returned to 63, and the pump stroke and water level in the overflow bottle were also brought back practically to the original conditions of the experiment. The pulmonary vein clamp was again tightened as in the two previous cases, with a shift in the air reaching the lungs, but of lower value than the one obtained in collection 2. It should, however, be noted that at this time the systemic blood pressure falls more than during the first period of compression, although the degree of clamping was identical, and that the pressure in the pulmonary artery does not rise so high. The animal does not compensate quite so well at the end of the experiment as in the beginning. The result emphasizes the fact that the degree to which air is kept out of the lungs by intravascular blood depends upon the existence of a powerful right ventricle coupled

with a fairly sound systemic circulation. Pulmonary edema will also exclude air but it is not a factor in these experiments.

3. Figure 9, experiment 3. In this case an experiment somewhat of the character of the last has been performed. During the first period of air collection the animal received air under a maximum pressure at the end of the down stroke of the pump of 5.8 cm. of water. Under these circumstances and with a pump rate of 63, practically all the air delivered actually reached the animal, there being little or no overflow. The pulmonary veins were now compressed with the usual rise in pulmonary arterial pressure and with a fall which gives way promptly to a rise in the systemic blood pressure. The exclusion of air from the lungs is moderate in amount, being 97 cc. On release of the compressing clamp there was a return to normal in so far as air distribution is concerned.

Between the eleventh and twelfth minutes of the experiment the level of the water in the overflow bottle was reduced from 5.8 cm. to 4.2 cm. Under these circumstances the elastic resistance to lung inflation is less completely overcome—there is a diminution of 1.5 cm. in the maximum pressure of air delivery—and in the fourth air collection, the normal period for this setting of the apparatus, there was less air in the expiration collection and more in the overflow collection. The pulmonary veins were now compressed to exactly the same degree as in the first period of compression. There was a greater rise in pulmonary arterial pressure and a fall followed by a rise in systemic blood pressure—a rise which began long before pulmonary vein compression was released. The fifth air collection taken during this period shows an exclusion of 169 cc. of air from the lungs, an increase in exclusion expressing the improvement in the circulation of the animal readily enough seen in the systemic and pulmonic blood pressures. After the compression air collection had been made and with the pulmonary arterial pressure still elevated, the level of the water in the overflow bottle was raised until all the air delivered at each stroke of the pump went into the animal—in other words, to a condition very similar to that existent during the first period of air collection in the experiment. It was found that the water level necessary to accomplish this was 6.8 cm. That is to say, the intravascular congestion produced by the clamping of the pulmonary veins, coupled with the ordinary elasticity of the lungs and chest, required a maximal intratracheal air pressure of 6.8 cm. in order to compress into the pulmonary air space the volume of air delivered by each stroke of the pump. The pulmonary vein clamp was released on the nineteenth minute of the experiment and after this release, which brought about removal of pulmonary congestion, it was found that all the air delivered by the pump reached the trachea and lungs when the water level in the overflow bottle was at 5.9 cm. In other words, the vascular congestion present during the height of pulmonary

vein compression necessitated to overcome it an increase in air pressure of 0.9 cm. of water. This is not a large amount and indicates the feeble character of the obstruction to air entrance offered by intravascular blood in the normal non-edematous and non-fibrotic lung tissue of the cat.

At the twenty-sixth minute of the experiment the level of the water in the overflow bottle was made 5.8 cm., the condition existent during the first three air collections in the experiment. Collection 7 was made and was followed by pulmonary vein compression of the same degree as in the two previous compressions. The blood pressure reactions are characteristic, and owing to the fact that the animal was in better condition than at the beginning of the experiment, as expressed by the higher systemic and pulmonic blood pressures, the amount of air excluded from the lungs by pulmonary congestion is increased, being 164 cc. as against 97 cc. in the first period of compression. On release of the compressing clamp there is a perfect return to normal as shown in the final air collection.

Discussion. The experiments we have described picture the readiness and the degree to which pressure in the pulmonary circuit can be increased by obstruction of the pulmonary veins, and they leave no doubt as to the fact that the degree of exclusion of air from the lungs produced by vascular congestion is directly related to the pressure under which the stagnated blood is held. In view of these simple and definite results, it seems strange that doubt can have existed as to the possibility of real increases in pressure due to obstructions placed appropriately in the pulmonary circuit. Our first experiments upon the influence of pulmonary congestion on air entrance into the lungs did not make use of pressure measurements, and we, too, were under the impression that the enormous size of the pulmonary vascular bed and the comparative weakness of the right ventricle precluded the possibility of noteworthy pressure increase. It is also true that if one takes pressure in the pulmonary artery by methods involving an open chest and very considerable mutilation of the animal, while the initial pulmonary arterial pressure may be practically normal under conditions of low blood pressure and shock, very little and at best only transient increases in pulmonary pressure will be seen when such operations as pulmonary vein clamping are employed.

The relation of cardiac inflow to pulmonary pressure and to the amount of blood in the pulmonary circuit are matters now under investigation in this laboratory. When these points are known and are coupled with measurements of pulmonary arterial pressure it will be possible to form a fairly complete picture of the degree and manner in which intravascular blood affects pulmonary ventilation. The data now presented merely indicate that for the occurrence of any considerable degree of air occlusion

a competent right ventricle and a good return of blood to the heart are essential.

SUMMARY

1. New apparatus for artificial respiration is described.

2. A table of measurements of pulmonary arterial pressure in the cat is presented.

3. By the use of experimental protocols the rise in pressure in the pulmonary artery accompanying vein compression is given and is correlated with the degree to which air is excluded from the chest by the existent pulmonary congestion.

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DIFFERENCES IN BLOOD PRESSURE IN THE ARM AND LEG IN NORMAL SUBJECTS

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Received for publication December 12, 1924

The systolic blood pressure has long been supposed to be the same in the brachial and femoral arteries in normal human subjects, though considerable differences have been recognized in patients with aortic regurgitation (1), arteriosclerosis (2) and hyperthyroidism (3).

At the suggestion of Dr. H. C. Bazett, we have endeavored to determine whether there might not be demonstrable, even in normal subjects, a "differential" pressure in favor of the femoral (though less marked than in the above-mentioned clinical conditions), if a more accurate photographic method were devised for measuring systolic pressure.

The apparatus used consisted of compressing bands 5 inches broad for the arm and 8 inches broad for the thigh, and recording bands of about 3 inches breadth each, all of the ordinary Riva Rocci type. The compression bands were connected with an air pump, mercury manometer and membrane manometer, and with one another. The recording bands were applied to the elbow and popliteal space, and were connected with sphygmoscopes (4) through which pressure changes due to pulse waves were transmitted to Frank capsules. The pressure in the Frank capsules could quickly be lowered to zero by opening a key controlling tubes leading to the open air (see fig. 1). The sphygmoscopes enabled a moderate pressure to be used on the recording bands so as to obtain good pulsations, without undue pressure being exerted on the Frank capsules. If the pressure changes swung the light from the capsules off the camera, it could be quickly brought back by opening the controlling key as mentioned above.

The membrane manometer recorded actual variations in pressure in the compression system, while the mercury manometer was only used to standardize the other. This was accomplished by platinum wires led into the mercury manometer, so that the circuit of a signal magnet was made or short circuited at 50 mm. and 150 mm. of mercury pressure. The zero point of the membrane manometer was marked by a small wire which threw a shadow coinciding with that of the membrane manometer lever at zero. The membrane manometer was Porter's modification of Hürthle's instrument with a light straw lever of about 3 inches length.

The subject was placed on a cot beside the blood pressure apparatus so that he could be at absolute rest and any influence of gravity would be excluded. After resting for about fifteen minutes the bands in the leg and arm were inflated several times, and records were taken after the subject had got used to this procedure. The subject then exercised while reclining on a cot, by lifting weights with the arm and leg which were not being employed for taking the record. For the leg a weight of 8.6 kilos was used and for the arm a weight of 3.2 kilos, and they were lifted simultaneously at the rate of about once in three seconds. The exercise was con-

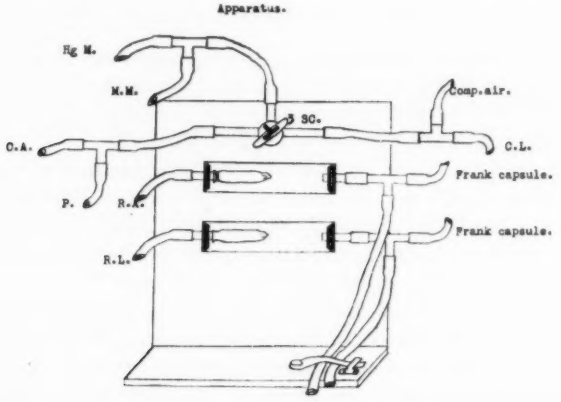

Fig. 1. *Hg M.*, to mercury manometer; *M. M.*, to membrane manometer; *C. A.*, to compression band on arm; *P.*, to pump; *R. A.*, to recording band on arm; *R. L.*, to recording band on leg; *3 SC.*, three way stopcock; *C. L.*, to compression band on leg. Comp. air, alternative connection to compressed air supply.

Two separate lights were used. The smaller of the two was directed against the mirrors on the Frank capsules. The larger was employed to cast a gray background and to throw shadows on the film of the recording levers of the membrane manometer and signal magnet.

The mercury manometer was arranged so as to record on the signal magnet, when the blood pressure was at the levels of 150 and 50.

tinued until the subject became fatigued, which took roughly from ten to fifteen minutes, varying with the subject. A record was started with a minimum loss of time after completion of the exercise.

The results obtained are given in the following table and sample records are reproduced in figures 2, 3 and 4.

The table only contains figures derived from a few definite records; many other records were also taken, and gave similar results, though the figures could not be read so certainly. In reading the records, in order to avoid any personal bias, the systolic pressure was taken to be that at which

TABLE I

CONDITION	PULSE RATE PER MINUTE	BLOOD PRESSURE IN ARM	BLOOD PRESSURE IN LEG	DIFFERENCE	
			Subject R		
		mm.	*mm.*	*mm.*	
Rest I	72	87*	148	60	
Rest II	72	94*	150	56	Average difference in rest = 58 mm.
Exercise I	90	112	192	80	Average difference in exercise = 83 mm.
Exercise II	93	118	205	86	Increased difference = 25 mm.
			Subject C		
Rest I	77	96	142	46	
Rest II	82	109	136	27	Average difference in rest = 37 mm.
Rest III	78	110	148	38	Average difference in exercise = 57 mm.
Exercise I	88	131	187	57	Increased difference = 20 mm.
			Subject B		
Rest I	72	108	149	41	
Rest II	80	108	138	30	
Rest III	72	117	151	34	
Rest IV	72	114	154	40	
Rest V	72	103	137	34	Average difference in rest = 36 mm.
Exercise I	84	136	188	52	Average difference in exercise = 72 mm.
Exercise II	84	130	222	92	Increased difference = 36 mm.
			Subject G		
Rest I	72	110	142	32	Average difference in rest = 35 mm.
Rest II	75	116	154	38	Average difference in exercise = 43 mm.
Exercise I	96	139	182	43	Increased difference = 8 mm.
			Subject E		
Rest I	72	96	118	22	

Summary

	mm.
Average difference in exercise all cases	68
Average difference in rest all cases	38

* These figures are rather low and probably some blood passed at the previous pulsation though not very definitely recorded. This would bring these values to 92 and 100 mm.

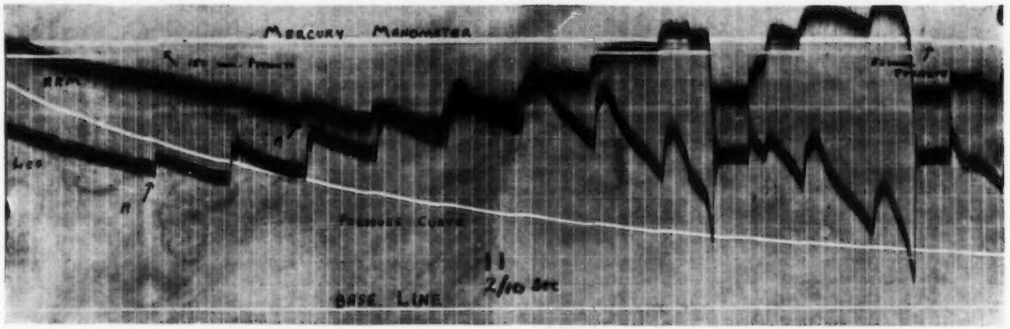

Fig. 2. Record of subject at rest (B rest III in table). Pressures read at points marked *A*. Upright lines mark $\frac{1}{5}$ second intervals. (Reduced to 40 per cent of original size.)

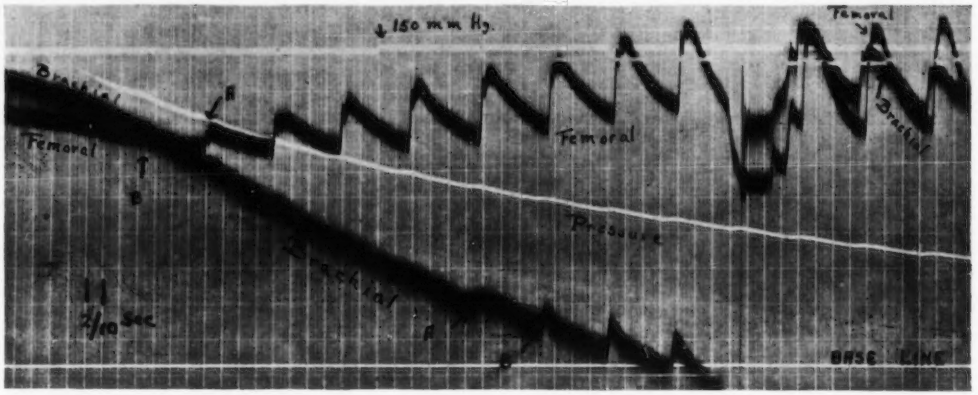

Fig. 3. Record of same subject as in figure 2, but after exercise (1). Femoral should probably be more correctly read at higher point marked *B*, and brachial could conceivably be read at lower point marked *B*. The readings are

$$\frac{\text{Femoral A } 188}{\text{Brachial A } 136}, \frac{\text{Femoral B } 205}{\text{Brachial A } 136}, \frac{\text{Femoral A } 188}{\text{Brachial B } 127}, \text{ and } \frac{\text{Femoral B } 205}{\text{Brachial B } 126}.$$

The difference of 52 mm. given in the table is therefore no exaggeration—more probably it was 69, and 52 is the minimum reading possible. (Reduced ¼.)

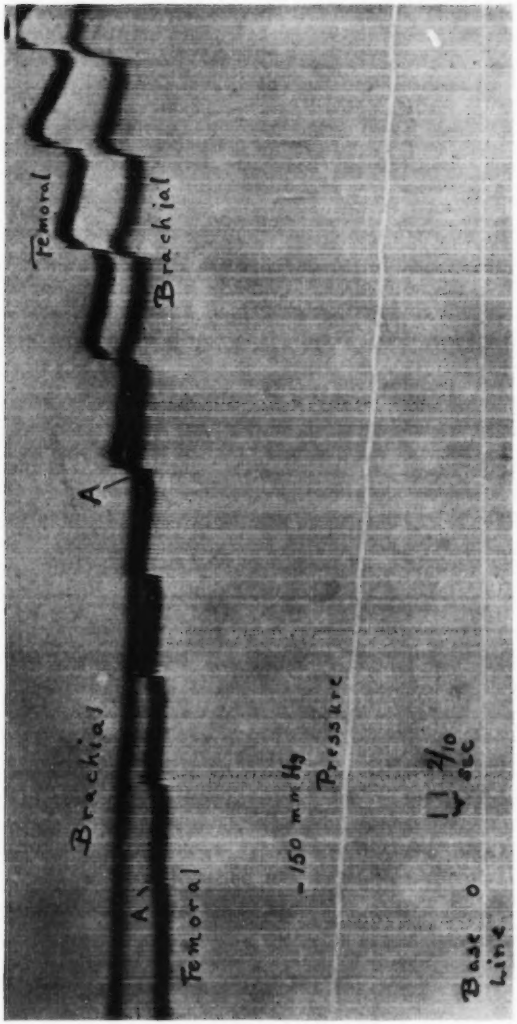

Fig. 4. Record subject E (see table) at rest with slower rate of deflation of cuff. (Reduced ⅓.)

the first quite definite pulsation could be seen. Actually the systolic pressure must have been higher than this. The actual pulsations from which readings have been taken are marked in the records of figures 2, 3 and 4 by *A*. It will be noticed that in figure 2 the brachial pressure might reasonably be read at one beat earlier, which would make the brachial pressure 134 instead of 117, and reduce the resting differential pressure on this occasion from an estimated 34 mm. to one of 17 mm. Quite big variations in the amount of difference between the two vessels can therefore result from only slight differences in the reading of the record, owing to the rapid rate of deflation of the compressing band, and consequently no undue emphasis can be placed on the actual values. On the other hand the deflation had to be carried out rapidly, especially after exercise, to obtain readings as nearly simultaneously as possible.

In the experiment shown in figure 4, the pressure was reduced very much more slowly so as to make the measurement of the difference in pressure more accurate. It will be seen that in this case the femoral pulsations were recorded for four beats before the brachial pulsations became evident and in no way could the record be read to reduce this to less than 3 beats. Even the latter improbable reading would leave the brachial pressure at 104 mm., and a difference in pressure of 14 mm.

In the experiment shown in figure 3 representing subject B after exercise, very slight pulsations in the brachial record are just detectable many beats before the pulsation marked *A*. These slight pulsations were usually detectable in both the brachial and femoral records at pressures much above the apparent systolic pressures, and they could be distinguished from those caused by blood passing the band, in that the latter, once they occurred, showed a progressive increase as the pressure in the compressing band fell toward diastolic pressure. The other small pulsations were probably due to vibrations of the limb on its rest, caused by the impact of the blood against the compressing band.

Whatever the errors concerned, in not a single record was there any doubt that the femoral pressure was above the brachial, even though it was much more difficult to get good records of the popliteal pulsation than of the brachial. Rarely was there any question as to the point at which pulsations started except between two pulse beats, and the variation in the degree of difference measured (according to which of these is taken) is rarely great (compare table, subject R). On several occasions the blood pressure measurements in the arm under resting conditions were checked by connecting an additional armlet to the compressing system, and reading the pressure by auscultation at the same time that the record was being taken. In every case the pressure determined by the recording system was above that read by auscultation, so that the results obtained depend probably mainly on the employment of a method more sensitive than

those previously in use. In particular auscultatory methods are probably very unreliable in the popliteal space.

Individual variations in the differential blood pressure were observed from day to day. Exercise increased this difference in blood pressure from 8 to 36 mm. (23 to 100 per cent), with an average increase in all cases of 30 mm. (63 per cent of the resting difference). Our method demonstrates absolutely the existence of this difference in pressure even in the normal subject at rest, and no variation in these results could be obtained by varying the degree of distention of the recording bands. The actual measurement of the difference may be exaggerated particularly after exercise, since the femoral record is necessarily obtained before the brachial (owing to the difference in their pressure) and the pressure in the femoral may have dropped slightly by the time the brachial comes through. The actual time between the occurrence of the pulsations in the two vessels never exceeded 13 seconds and averaged 7 seconds, since to reduce this error as far as possible the pressure was allowed to fall rapidly in the compressing bands.

The results are not readily explained by L. Hill's hypothesis of a contraction of the femoral artery (1), since these figures were obtained on normal subjects, lying at rest, and both limbs were compressed and decompressed an equal number of times before the readings were taken. After exercise again, when the difference was greater, no different degree of contraction of the vessel walls between the two limbs can be supposed, since neither of the limbs used for recording was exercised, and yet the exercise employed both the upper and lower parts of the body.

On the other hand, these figures bring the circulation in man more in line with that of experimental animals in whom the systolic pressure in the femoral, recorded photographically, is commonly found to be greater than that in the carotid. The increased difference after exercise is explicable, if it is due to the conversion of kinetic energy into pressure by the blocking of the blood flow by the compressing band, according to the theory advanced by Bazett (5).

CONCLUSIONS

1. A photographic method is described for the simultaneous registration of systolic blood pressures in both arm and thigh in man. Comparison is rendered more reliable by the connection of both compressing bags together, so that they are blown up by the same pump, and recorded on the same manometer.

2. Using this method the systolic pressure in the leg is found to be constantly higher than that in the arm even in the normal subject lying down at rest, the difference being commonly some 20 to 40 mm. of Hg.

3. Immediately after exercise this difference is increased, differences as

high as 92 mm. being sometimes found. After exercise this averaged 68 mm. in subjects giving an average difference of 38 mm. at rest.

4. The increased difference after exercise is unlikely to be due to any local change in the vessels, since the exercise taken involved both upper and lower limbs, but not those from which the records were obtained. These results therefore do not support L. Hill's theory of the causation of a differential pressure, but are in agreement with Bazett's theory of the importance of kinetic energy in its production.

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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

Thirty-Seventh Annual Meeting

Washington, D. C., December 29, 30, 31, 1924

Conduction without progressive decrement in nerve under alcohol narcosis. Hallowell Davis, Alexander Forbes, David Brunswick and Anne McHenry Hopkins, Harvard University.

It is well known that narcotics, such as ether and alcohol, when applied to a nerve trunk depress its activity and eventually suspend conduction entirely. Since the blocking effect of such treatment by these narcotics has repeatedly been found to depend not merely on the concentration of the drug but also on the length of nerve subjected to its effect, it has been inferred that under narcosis conduction occurs with progressive decrement, the impulse becoming gradually smaller in the narcotized region. On the basis of this inference many and various experiments have been devised and performed and important conclusions as to nerve function drawn from them; notably the all-or-none law. Recently an apparent conflict between fact and theory as regards the velocity of subnormal impulses[1] led us to re-open the whole question of conduction with a decrement. The electric response, or action current, which seems to be an essential feature of the nerve impulse, and which the accumulated facts in support of the membrane theory have led us to consider an integral part of the mechanism of conduction, affords the only direct measure whereby we can make an accurate quantitative determination of the size of the impulse at a given point in the nerve. We, therefore, set out to determine whether the action current does or does not grow progressively smaller as the impulse traverses a region of uniform narcosis.

Our method consists in laying a peroneal nerve of a cat in a chamber (fig. 1) which allows alcohol vapor to act on a portion of it only, while we stimulate outside the narcotized region at *S* and lead off the monophasic action current to a string galvanometer, *G*, from electrodes *1*, *2* and *3* within the narcotizing chamber and from *4* beyond it on unnarcotized nerve. The circuit is completed through an indifferent electrode at *5* in every case. The control at electrode *4* is essential to prove that the narcotic has not established a total block in some of the fibers and that therefore such changes as are found within the chamber represent changes in the individual fibers. Failure to make this control renders worthless numerous previous experiments on the action current in the narcotized region. Measurements of resistance in the nerve have been made and the experiment controlled with respect to them. After recording the normal action current at each lead we allow the alcohol vapor to flow through the chamber and record the action current from all the leads at frequent intervals. The results show that although the action current is not reduced at

[1] Forbes, Ray and Griffith: This Journal, 1923, lxvi, 587.

lead 4, indicating that in none of the fibers has the impulse been blocked, the action current within the narcotizing chamber becomes steadily smaller as time progresses, and that the relative depression is approximately equal at all points in the narcotized region; in short, there is no progressive decrement.

After we had completed these experiments, we learned that Kato[2] and his collaborators in Tokyo had approached the same problem from a slightly different angle and performed essentially the same experiment, using amphibian material, with precisely the same result. Furthermore, Kato and his collaborators found the sources of error which had led to the inference of conduction with progressive decrement, effects due to the

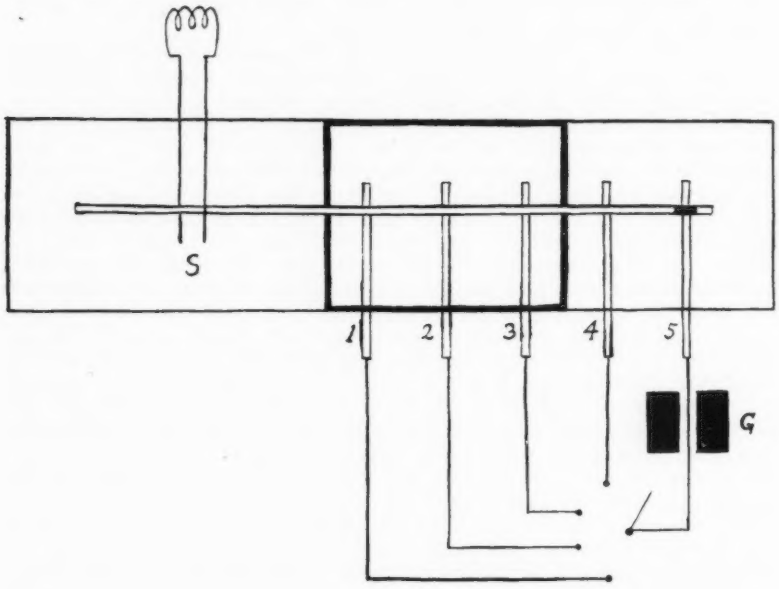


Fig. 1

diffusion of the narcotic near the edge of the narcotizing chamber and to the spread of stimulating current.

Besides the main conclusion that the nerve impulse in a region of uniform narcosis is not conducted with progressive decrement, but is reduced almost immediately to a level which at each point depends on the depth of narcosis, we should also note that, since the size of the impulse depends only on the condition of the nerve at each point and not on its previous history, it follows that the energy of the impulse comes not from the stimulus but from the fiber, and the all-or-none law is more firmly established than ever.

[2] Kato, G.: The theory of decrementless conduction in narcotised region of nerve. Nankodo, Hongo, Tokyo, Japan, March, 1924.

Response of a muscle fiber-group to apparent stimulation of a single motor fiber in the nerve trunk. FREDERICK H. PRATT, Boston University.

Excitation of a nerve so as to be selective for its fiber elements can be more or less successfully achieved by each of two methods. The first, in which the whole nerve is stimulated, is based upon the different thresholds of the several fibers, and their relative position in the path of current. A second selective method of stimulation depends upon the actual application to the responding unit of a shielded region of relatively extreme current density.

In recent observations the following forms of active electrodes have been used for stimulating the sciatic or its tributaries in the double leg preparation of the frog:

A platinum wire polished to a microscopic point is covered with glass fused and retracted over the tip in an invisible layer; the tip is pierced with an electric spark, giving the smallest shielded stimulating area obtained—apparently less than 1μ diameter. A similar electrode may be made with a sewing needle coated with cement and similarly spark-pierced, which affords a less minute opening. Finally, sharpened pore electrodes, pore diameter 1 to 2μ, have been used successfully.

Results with all these forms of electrodes are closely similar. The active electrode is introduced through a slit in the nerve, its point lying among fibers teased with fine needles. Minimal make or break shocks may now be sufficiently localized to bring into play small portions of the various muscles, a single active area in but one muscle being characteristic. The secondary coil may in some cases be moved 6 or 8 cm. without change in size or area of response; sometimes a rheostat governing the primary current may be covered to nearly its full extent. More frequently a secondary coil range of 1.5 to 2 cm. limits the stimulus gradient between zero contraction and abruptly augmented contraction.

Certain observations suggest that such response is the result of single nerve fiber stimulation. 1, As a rule the response is incapable of diminution except to zero. 2, It may involve an area comparable with that which would be occupied by the estimated muscle fiber organs of the nervous conductor. 3, It is incapable of increment except abruptly. Often, upon increase of current, it is suddenly accompanied by minimal activity of some distant muscle without itself showing augmentation.

It is desirable to have a method whereby not only muscle, but nerve-muscle, may be studied on one step of the discontinuous series of values that make up the minimal-to-maximal range of response. To do this it is necessary to attain a working range of stimuli without the disturbance of new steps or of imminent descent to zero, the attainment of actual single-fiber response being of secondary importance. It is also desirable to have, as above described, a preparation readily obtained, quick to prepare, and simple to manipulate under the recording microscope. In the muscle as a whole such phenomena as the mechanical effects of tetanus, tonus, staircase and fatigue all may be obscured or misinterpreted; for the accession or elimination of fiber activity may alone simulate each one of them. But in the single step preparation such phenomena, insofar as they exist, have a clear field to reveal themselves. They appear as continuous functions if at all, unobscured by the discontinuous gradients dependent upon the stimulus or threshold variables.

Characteristics of tetanus in the reduced muscle. FREDERICK H. PRATT, Boston University.

Superficial fiber groups of the frog's sartorius are tetanized with currents which, singly, produce a given size of twitch. The resulting curve shows a prompt assumption of the twitch level, which may persist on the step involved or give way to the successive assumption of steps corresponding to higher twitches. Fatigue now manifests itself in a subsidence by regressive stages to the lower levels. This suggests that an important mechanism in the production of the increment of tetanus over twitch is the accession of new fibers or fiber groups, and that the instability in value of a submaximal tetanus is a reflection of instability of threshold in the fibers involved.

Tissue growth and vitamines. MONTROSE T. BURROWS, Barnard Free Skin and Cancer Hospital and the Department of Surgery, Washington University School of Medicine, St. Louis.

The B. tumefaciens when injected into the tissues stimulates an active growth of cells. These cells grow to form a dense, non-vascular cancerous tissue. Jorstad and I have tried the effect of feeding this organism. It acts as a ready and even more efficient substitute for vitamin B (yeast) in a diet containing no vitamin B. The growth stimulus of this organism allowed to act through the blood vessels produces a normal vascular and functioning tissue or a normal growth of the animal. Acting outside of the blood vessels it produces in contact with the tissue cells a cancerous organization. The two day old cultures which we have tested contain no detectable vitamin A.

Vital Function Studies. 1. A study of Dreyer's vital capacity standards in endocrine disorders. ALLAN WINTER ROWE, Evans Memorial, Boston.

As a result of measurements, Dreyer has formulated certain interrelationships between several biometric capacities and has established criteria for normal performance as an index of physical efficiency. The present study deals with an evaluation of the accuracy of the concept. The correlation of chest to sitting height (called by Dreyer trunk height) is found to be good. He expresses weight in terms both of sitting height and of chest circumference. In regard to the former we find relatively slight variations in the sitting height index, the average of a very large number of observations being approximately 0.525. The outside variations of this magnitude are found on the one hand in the achondroplastic dwarf, with a value approximating 0.56; and on the other, in the pituitary giant with a value approximating 0.48. The chest influence, on the other hand, is very great and as Dreyer's values are based upon individuals of normal configuration, with the excessively thin and obese extrapolations leading to absurd magnitudes are the result. For example, a woman 150 cm. tall is by the combined Dreyer standard 5 per cent below weight, by the trunk height standard alone plus 170 per cent above weight, when actually weighing 129.5 kilos. On the other hand, a woman of 166 cm. and weighing 45.8 kilos is 6 per cent overweight by the combined standards, and 27 per cent below weight by the trunk alone. Obviously, the Dreyer comparison gives no index of the person's physical habitus in relation to their normalcy, but does give a means of an approximate calculation of body weight for those who are not able to be placed on a weighing machine. This is of great

potential value in bedridden cases where a basal metabolism determination is desired and where the weight is essential for the establishment of the calculated norm. To permit the use of Dreyer's trunk height value, the writer has determined the relationship of the perineum-crown length in a recumbent position to the sitting height. It is found that the former measurement is 1 cm. less than the latter in women and is 1.8 cm. greater in men; this presumably is due to the different pelvic configuration. Since many cases fail to show satisfactory results in calculating the weight, because of the extreme variations introduced by the chest extrapolation, the writer has sought the relationship between maximum hip circumference and body weight. Two equations have been established as the result of a large number of observations on men and women of approximately so-called normal configuration. These equations are:

$$\text{Weight}_{\text{Male}} = 1.616\ \sqrt[0.426]{\text{Hips}},\quad \text{Weight}_{\text{Female}} = 1.387\ \sqrt[0.429]{\text{Hips}}$$

It is found by use of the combined trunk height and hip circumference standards that a very much closer approximation of observed and calculated weights can be obtained than where the Dreyer chest factor is used.

Dreyer's third criterion is his so-called vital capacity, by which he means lung capacity. He establishes three standards of performance on the basis of physical habit and occupation. In the writer's opinion, a far better practice would be to establish a single standard and then allow wider limits of variation, for the factors mentioned above. Measurements made on healthy young men and women in this country show that Dreyer's Male A standard and Female B standard conform most nearly to our conditions. The lung capacity is referred to three criteria: trunk height, weight and chest girth. Again, the chest measurement exercises an undue influence on the magnitude of the final average, while the weight factor falls in the middle distance. Only those measurements referred to sitting height are to be regarded as dependable. West has also established a lung capacity relationship, with both standing height and with area. The weight element influences this latter criterion somewhat, but there is an excellent correlation between Dreyer's trunk height and the average of the two West comparisons.

A large number of endocrine cases have been studied in connection with these measurements. Cases showing the clinical stigmata of endocrine disturbance, but which on analysis were found to be non-endocrine in character, show an average failure in the vital capacity of minus 17 per cent. Substantially the same figure is obtained with pituitary cases. Thyroid cases show an average failure of minus 28 per cent, while the gonad group falls between these values at minus 24 per cent. The individual variations, however, in each of these groups rob the vital capacity measurements, considered alone, of any diagnostic significance. The averages may be said to represent tendencies only.

The effect of insulin on the oxygen consumption of certain marine fish and invertebrate. J. B. Collip, Marine Biological Station, Nanaimo, B. C.

During the summer of 1924, an opportunity was afforded to study the effect of insulin on the oxygen consumption of certain marine fish, crabs and clams. Oxygen was determined by the Winkler method. The results on the whole were quite negative. Several hypoglycemic reactions were

observed in fish injected with insulin. These developed as a rule within 24 to 48 hours after the administration of the insulin. If a reaction occurred while an oxygen consumption determination was being made, a sharp rise occurred in the rate of utilization of oxygen. This phenomenon was noted on serveral occasions.

Clams and crabs seemed particularly resistant to insulin injections. No appreciable change in the oxygen consumption was noted after insulin injections in these forms.

A parathyroid hormone and its physiological action. J. B. COLLIP, Department of Biochemistry, University of Alberta, Edmonton, Canada.

By methods, which have been elsewhere described,[1] an extract, which it is believed represents in potent form and a fair degree of purity the essential hormone of the parathyroid gland, has been prepared. It has been found that tetany in parathyroidectomized dogs can either be prevented or controlled by the administration of this extract by subcutaneous injection. A heavy meat diet has in no wise affected the results in this regard. Also by the use of this parathyroid preparation, parathyroidectomized dogs have been kept in excellent condition for long periods of time. Such animals will as a rule manifest severe tetany after withdrawal of treatment. In this way treated animals may be proven up from time to time even many weeks after the removal of the glands. One such animal is now in perfect health some ten weeks after the operative removal of the thyro-parathyroid apparatus. This animal has been allowed to develop severe tetany on numerous occasions and invariably an injection of the special extract has restored the animal to normal within three hours.

It has been shown that the administration of this extract to normal dogs is followed by a rise in the level of blood serum calcium. The normal dog may therefore by used to test extracts of parathyroid glands for potency and there is a possibility that a definite physiologic unit may be arrived at in this way. It has also been ascertained that the changes induced in the calcium values of blood serum of normal dogs by injection with this extract follow a fairly definite curve. This is also true of the parathyroidectomized animal. These latter are somewhat more sensitive to the extract providing they are not in tetany.

Varying doses of the extract have been administered to a number of normal dogs. A small dose has thereby been shown to have almost the same effect on the blood serum calcium curve as a very large one. The curve in each instance extends over approximately twelve hours with the maximum effect being manifested between five and nine hours.

Successive doses of the extract have also been administered to a number of normal dogs. Two factors have been varied in these experiments, one the size of dose, the other the time interval between injections. It has been found that the former is of relatively little importance, so long as an effective dose is used, while the latter is of the greatest importance. By successive injections of the potent extract at intervals of twenty minutes to eight hours, a condition of profound hypercalcemia has been produced, values up to 21.5 mgm. per 100 cc. having been observed. This phenomenon is explained by cumulative action resulting in the pyramiding of the effect of each successive injection upon the last.

[1] Collip, J. B.: Journ. Biol. Chem., in press.

Typical symptoms are manifested by dogs in a condition of extreme hypercalcemia induced by overdosage of the parathyroid extract. These may end in death or complete recovery may occur. Vomiting is one of the first symptoms to be observed. This is followed by profuse diarrhea. The animals become very weak and there is general atonia. The circulation becomes impaired and in the pre-terminal stages it is a matter of great difficulty to secure blood samples. Slight dyspnea has been observed in some animals. In the fatal cases there is as a rule bleeding into the gastro-intestinal canal and blood stained fluid is usually both vomited and passed by bowel shortly before death ensues.

Profound changes have been observed in both the physical and chemical characteristics of the blood in animals in a condition of extreme hypercalcemia. These changes have been most pronounced in the terminal state. The results obtained in fatal cases may be summarized briefly as follows: 1 great increase in viscosity; 2, 15 to 20 per cent increase in osmotic pressure; 3, increase in phosphates 100 per cent or more; 4, increase in non protein nitrogen as high as 400 per cent; 5, increase in urea nitrogen as high as 400 per cent; 6, increase in protein; 7, diminution in halogen, 10 to 15 per cent; 8, diminution in alkali reserve; 9, diminution in blood volume 5 to 15 per cent.

This hormone is therefore capable of producing most profound changes in the mineral composition of the blood. It will consequently have influence upon water balance, hydration of protein, viscosity and undoubtedly other factors as well.

This extract has already been injected into a number of human individuals and the blood calcium has been observed to undergo an increase. The extract has been used in case of infantile tetany[2] and in this case a remarkable result was obtained. The action was absolutely that of a specific in this instance. It has also been used with excellent results in a case of acute nephritis with extensive edema in a child of five.[3] In two cases of nausea and vomiting of pregnancy[4] in which it has been used, definite improvement has been noted.

The somewhat similar signs manifested by parathyroidectomized dogs in the pre-tetany state and certain cases of typical exophthalmic goiter suggested that this latter condition might be associated with hypo-function of the parathyroid glands. The blood calcium in three such cases has been determined and the values were 8.5, 8.2, and 10 mgm. per 100 cc. The special extract was used in the first case.[5] The basal metabolism was + 77 before treatment was started and in 9 days it was gradually reduced to −11. The blood calcium was found to be 10.2 on the second day after the extract was started.

No claim for the clinical effectiveness of this extract in conditions other than those with definite hypo-functions of the parathyroid gland is made at this juncture. The results so far obtained are suggestive only. The one claim, which is made however, is, that herein is an hormone in potent form derived from the parathyroid glands of the ox which will definitely raise the level of blood serum calcium both in animals and in man. It is a specific for tetany of hypo-parathyroidism. It is probable also that this hormone will be of use in any condition in which $CaCl_2$ is of benefit.

[2] Collip, J. B. and D. B. Leitch: Can. Med. Assoc. Journ., in press.
[3] By courtesy of Dr. D. B. Leitch.
[4] By courtesy of Dr. J. Baker and Dr. C. MacDonald.
[5] By courtesy of Dr. W. H. Scott.

Thyroid tolerance following mild experimental hyperthyroidism. O. O. STOLAND and LILIAN DONALDSON, University of Kansas.

Mild hyperthyroidism was developed in albino rats by administration of non-toxic doses of desiccated thyroid for a period of four months. Control groups were weighed, fed, and otherwise treated as the experimental animals. Male and female controls and experimental groups were maintained separately. A certain number of the controls and the fed animals were then subjected to doses of thyroid known to be toxic and the effect determined by the growth curve. The experimental animals developed a mild degree of hyperthyroidism as shown by the fact that their weight curve was only slightly lower than that of the controls. The toxic doses produced the same degree of effect upon the controls as upon the fed animals, as indicated by the loss of weight. Of the males a larger number of the hyperthyroid animals died from the toxic doses than did the controls. The reverse was true of the females. The loss of weight from the toxic doses of thyroid is less in females than in males indicating a greater susceptibility to the thyroid hormone in males than in females. Our results indicate that the tolerance of rats to thyroid hormone is not altered by a mild degree of hyperthyroidism in females, but male rats become more susceptible to this hormone.

Production of albuminuria by renal vasoconstriction. ISAAC STARR, JR., Department of Pharmacology, University of Pennsylvania.

In rabbits the continuous intravenous infusion of small concentrations of adrenalin for 5 to 15 minutes is followed by transient albuminuria.

In eviscerated dogs under luminal a prolonged injection of adrenalin causes decrease of kidney volume during the injection which is followed by a transient albuminuria. Partial occlusion of the renal artery produces a similar result. When adrenalin causes an increase in kidney volume, no albuminuria follows. Temporary renal vasoconstriction caused by inhalations of CO_2 or by bleeding and later replacing the blood is followed by transient albuminuria. If the kidney vessels constrict spontaneously and later are dilated by diuretics, a similar transient albuminuria follows.

Cats frightened by tying them on their backs and catheterizing, by a dog, or by weak electric shocks have a temporary albuminuria afterward. After the cat becomes accustomed to the procedure, the albuminuia no longer appears.

These effects occur in kidneys grossly and microscopically normal.

The results are consistent with the conception of Richards, 1922, that renal vasoconstriction by depriving certain glomeruli of their circulation for a sufficient time would damage them so that, when blood flow through them is resumed, the blood proteins pass into the urine.

Staining of renal tubules by intracapsular injection of various dyes. J. M. HAYMAN, JR., Laboratory of Pharmacology, University of Pennsylvania Medical School.

Since Heidenhain's experiments many students of renal physiology have considered the presence of dye in the cells of the convoluted tubules following intravenous injection, as evidence favoring the secretory function of these cells. Others have inclined toward Sobieranski's opinion that the staining might result from reabsorption of a dye-containing filtrate from the lumen of the tubule.

Evidence as to the validity of opinions based on the histological picture of fixed sections of the kidney after injection of dyes into the blood stream was sought by comparing the results obtained with frogs after intravenous (c$^{\text{a}}$ lymph sac) injection with those following introduction of a dye directly into the lumen of a tubule by injecting it into Bowman's space. The intracapsular injections were made by Wearn and Richards' method. Indigo carmine, sodium carminate, methylene blue, trypan blue, toluidin blue, phenolsulphonephthalein and iron salts were used. Intravenous and intracapsular injections yielded similar staining of the tubule cells in all cases. All the dyes tested are present in the glomerular fluid following intravenous injection. It is believed that the presence of dye in the tubule cells following intravenous injection is no better evidence of secretion than of reabsorption, and that possibly it is evidence of neither.

Alkalosis produced by ingesting urea. EDWARD F. ADOLPH, The Johns Hopkins University.

When urea, ammonium bicarbonate or ammonium citrate were taken by mouth into the human body, it was found that the alkalinity of the blood became significantly greater. This shift in the acid-base equilibrium was exhibited in the increased CO_2 tension of the air in the lung alveoli, and in the decreased hydrogen ion concentration and titratable acidity of the urine.

The normal alveolar CO_2 tension was determined several times daily for a month. At no time did the tension change more than 1.1 mm. of mercury during a single day (using the average of an inspiratory and an expiratory sample obtained by the Haldane-Priestley method), even when water or sodium chloride was ingested; and it varied only between 40.9 and 42.7 mm. during the entire month. But when urea or organic salts of ammonia were ingested in 6 experiments, the alveolar CO_2 tension rose 0.9 to 2.4 mm. in 4 of them; remained constant in one of them; and fell 3.5 mm. in one of them. In every case the actual acidity of the urine, measured colorimetrically, was parallel. In the first 4 experiments mentioned above an alkalinity developed; in the other 2 the urine remained acid. The urine was studied in 7 additional experiments, and in all but 2 of them it became alkaline. Thus the acid-base equilibrium was disturbed by the ingestion of the nitrogenous compounds in 10 out of 13 experiments, and was shifted toward alkalinity in 9 of these 10.

The mechanism by which alkalinity is produced by these compounds is not to be sought in the properties of urea itself. Momose, working in Barcroft's laboratory, showed that ingested urea and ammonium lactate affected the oxyhemoglobin dissociation curve exactly as alkali does, and that there is no effect when added to blood in vitro. In all cases the alkalinity is evidently due to the absorption of ammonia produced by bacteria in the intestine from the nitrogenous compounds. Thus, Barnett and Addis found a great increase in the ammonia concentration of the blood when large doses of urea were administered to rabbits, which are herbivorous animals. The absorption of ammonia into the portal blood system has been demonstrated also in dogs by Folin and Denis and by Henriques and Christiansen.

That bacteria are not the only agents for the conversion of urea into ammonia is indicated by the following observation. In each of the experiments in which strong urea solutions were ingested (usually 3 M), the

chemical sensation of ammonia was distinctly present in the mouth and lasted several minutes. Evidently in at least the sense tissues of the mouth urea hydrolyzes to a detectable extent. This observation led to a search for similar conversion by other animal tissues and fluids, including saliva, but none was found. There is, however, some recent evidence that such a conversion sometimes occurs in the mammalian kidneys, and it is well known what all tissues produce ammonia in catabolism and autolysis. It seems, therefore, that urea is a neutral substance in most tissues, and does not lead to ammonia formation merely by increase in its mass in them. But urea taken through the digestive tract or injected in such doses that it enters the digestive tract will usually produce an alkalosis which is probably due to the formation of ammonia.

It is worth raising the question whether the alkaline tide which follows meals is not partly due to the absorption of nitrogen as ammonia.

The chemical sensitiveness of the kidneys. EDWARD F. ADOLPH, The Johns Hopkins University.

Graphical analyses have been made of many of the published data in which the rate of excretion of a particular substance has been accurately compared with the blood concentration of the same substance at the time. Only after copious addition of some one substance to the body can a quantitative measure be obtained of its influence upon the kidneys. It was found possible to estimate *a*, the threshold value for each substance, and *b*, the increase in rate of excretion that corresponds to a known increment of the substance in the blood.

Urea is the substance about which we are best informed. Data on men, dogs and rabbits all show a direct proportionality between the rate of excretion and the blood concentration over an enormous range. It is also evident that a definite small blood concentration is necessary before the kidneys are excited to excrete the substance. Normally the blood concentration is near this value. Some excretion occasionally occurs below it, and this is due to the augmenting or washing-out activity of the water. It has been demonstrated that this water is carried through the kidneys chiefly in conjunction with chloride excretion. Thus urea has a definite threshold for kidney stimulation, and this threshold differs characteristically in the three species of mammals studied.

Chloride, acid and bicarbonate all show the same type of direct proportionality between blood concentration and rate of excretion. Their thresholds can be accurately found only by measurements made after the ingestion of the substance in question. For water the data on blood concentration are still insufficient for correlation. It is known, however, that there is a definite threshold, and that any dilution by water ingestion increases the water excretion.

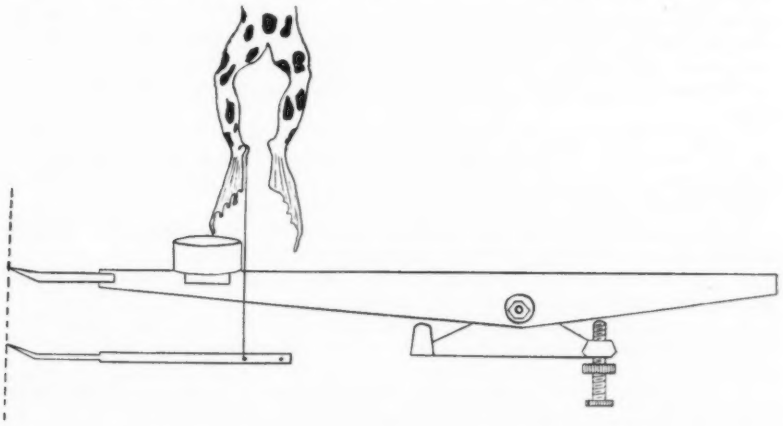
Substances such as potassium and phosphate appear to have thresholds in the sense that urea has one. Data upon them are insufficient for accurate correlation. Phosphate, ammonium, and probably other substances such as urea are in addition markedly influenced by the acid-base equilibrium of the blood. It may be noted that only those substances have high kidney thresholds which do not have a differential distribution between each tissue cell and its surrounding medium.

From the available data the following conclusions may be drawn: *a*, There is a range of blood concentrations for every substance within which

the kidneys are not stimulated to excrete it. This insensitive range represents a tolerance which effects a great saving in kidney activity. *b*, The response to concentrations above this threshold is immediate and without measurable sensitizing or acclimatizing influence. *c*, Each substance is excreted individually and specifically when present in a concentration above the threshold value. *d*, Below the threshold value there is a slight washing out of substances by the flow of water through the kidneys. *e*, Above the threshold value the rate of excretion is directly proportional to the blood concentration. Equimolecular or isosmotic quantities of different substances have different stimulating values. *f*, The rate of the kidneys' activity cannot be correlated with the amount of substance ingested, because the intestine and other tissues serve to buffer the changes in blood concentration. *g*, Ordinarily the kidneys never reach the limit of their ability to maintain the proportionality between blood concentration and rate of excretion, however high the former may rise. *h*, The thresholds and excretory ratios are probably modified by changes in the proportions of electrolytes in the blood. *i*, Other tissues which are highly sensitive to chemical changes in the fluids which bathe them, such as the respiratory center and certain glands, show similar phenomena of threshold and direct proportionality betweeen stimulating concentration and quantity of output.

An acid stimulator signal. D. J. EDWARDS, Cornell University Medical College, and C. J. WIGGERS, Western Reserve University Medical School.

Goltz's experiment for determining the reflex time elapsing after the immersion of a frog's foot into a 0.1 per cent solution of H_2SO_4 under normal and abnormal conditions or after the use of drugs (e.g., strychnine)



is incorporated in the class exercises of many physiology and pharmacology laboratories. As a rule class directions read to "measure accurately the reflex time" but no apparatus is placed at the students' disposal for even an approximately accurate determination of this interval. Rarely is even a stop watch available.

To provide a means of making more nearly accurate estimates of "reflex time" the acid stimulator signal was devised about 1917 and has been successfully used in class work in both the Physiology Laboratories of Cornell University Medical College and Western Reserve University Medical School since that time.

It consists of a rigid wooden beam 40 cm. long, shaped like a balance beam and moving on a fixed bearing so placed that the longer arm is 24 cm. and the shorter 16 cm. The longer arm is equipped with a writing point and mounts a small lead receptacle for the dilute H_2SO_4 shaped like a miniature bath-tub. A frog is suspended over this apparatus so that the foot which hangs just over the acid receptacle is further attached by a thread to a writing lever.

When the short arm is depressed the longer arm containing the acid receptacle is raised rapidly so that the foot is immersed. The moment of this immersion is directly recorded by a pointer attached to this arm. As the foot is withdrawn from the bath it raises the recording lever to which it is attached. The interval represents the reflex time.

The fundamental nature of premature ventricular contractions in the dog.

CARL J. WIGGERS, Western Reserve University Medical School.

It is generally considered as demonstrated that when electrical stimuli are applied to the normally beating amphibian or mammalian ventricle, the amplitude of the resultant contraction is determined by the interval elapsing after the end of the previous systole. To what extent this phenomenon is due to the interval of muscle recovery and to what degree to the extent of ventricular filling has not been clearly separated in the case of mammalian hearts. This has been partly due to the fact that until recently we have had no mechanical appliances capable of recording ventricular contraction in an accurate manner. In experiments designed to analyze these factors by the use of optically recording manometers, a number of interesting facts were established. While it is not my purpose to discuss them in detail at present, a few of them may be briefly indicated, viz.:

1. The entire interval of ventricular systole is not refractory to stimuli; on the contrary, stimuli applied 0.03 to 0.05 second before its end as indicated by the incisura of the aortic and ventricular pressure curves evoke a contraction during the isometric relaxation phase.

2. There is no evidence that such stimuli extend the duration of the existing systole or that they produce superimposed beats.

3. The earlier the stimulus is applied, the longer the apparent latent period becomes (range = 0.05 to 0.12 second).

4. The amplitude of contraction as gauged by the maximum intraventricular pressure developed is not solely determined by the lapse of time.

5. Occasionally, no contraction results when a normal impulse falls due after the completion of a premature contraction.

Before these and other reactions can be interpreted as specific peculiarities of heart muscle it is necessary to ascertain to what extent modification in the amplitude and duration of artificially elicited contractions represent a real index of the contraction processes occurring in individual fractions of heart muscle.

An analysis of the premature contractions of the ventricle stimulated

shows a number of deviations from the normal: 1, The isometric contraction phase is prolonged; 2, the gradient of the pressure rise is much slower; 3, the ejection phase is abbreviated, and 4, total systole is shortened. This abbreviation of systolic ejection and the consequent shortening of ventricular systole accords with dynamic laws laid down by the writer and his associates, viz., that the degree of diastolic filling and the initial tension not only modify the volume of systolic discharge but the duration of the ejection phase as well; the smaller the diastolic filling and the lower the initial tension, the shorter the ejection phase of effective beats becomes.

In order to compare the contraction processes in artificial and natural beats it therefore becomes necessary to compare such beats under conditions where initial pressure and diastolic filling intervals are equal. This was accomplished by completely stopping the heart by vagal stimulation and then applying artificial break shocks to the ventricle at rates equivalent to the normal.

Under such conditions the artificially elicited beats show *a*, a lower maximum pressure in the ventricles; *b*, a smaller systolic discharge; *c*, a lengthening of the isometric contraction phase; *d*, a lengthening of systolic ejection, and *e*, a prolongation of the entire systole.

The possibility that these changes may be due to *a*, direct vagal effects on the ventricle, *b*, absence of auricular contractions, has been excluded.

The conclusion is arrived at, after careful consideration, that the changes are due to an abnormal spread of the excitation of such an order that the beat starts as a local fractionate contraction process arising at the point stimulated and spreading more or less radially from fraction to fraction and is continued by a more synchronous contraction of the remaining ventricular fibers excited via the bundle branches.

It is obvious therefore that changes in the amplitude and duration of premature contraction in the mammalian ventricle can not be used as an index of the contractions processes of the individual fractions making up the ventricles, and that conclusions as to the fundamental reactions of the heart muscle must be drawn with considerable reserve and caution.

Observations on the function of the frog's kidney. MARIAN M. CRANE (by invitation), Johns Hopkins Medical School.

Observations have been made on the normal function of the frog's kidney. It was possible to make this a more direct and complete investigation of the subject than has previously been reported because of the large size of the frogs used (rana catesbiana), which varied from about 500 to 900 gm. in weight. In nearly every case, the experiments were carried out on the normal animal without operation or anesthesia. Urine was collected over comparatively short periods by catheterization of the bladder, and blood samples were drawn from the heart by means of a syringe and needle inserted through the skin. Diuresis was developed by injection of distilled water into a lymph sac. Sometimes intravenous or subcutaneous injections were used to introduce substances into the animals, while in other experiments the frogs were placed in various solutions and allowed to absorb the substance.

Chlorides, bicarbonates and glucose were found in normal urine only in traces or minute amounts much smaller than the concentration in the blood. Phosphates are also present in very small quantities but may be concentrated to slightly more than the blood value. The same is true for uric

acid. Urea on the other hand may be concentrated anywhere from 2 to 10 times under normal conditions, and when the frog is drying much higher values (up to 65 times) are reached. Phenol red when introduced into the blood stream is concentrated as effectively as urea.

When the blood chloride concentration is increased above 225 mgm. per 100 cc. (normal values are from 150 to 225 mgm. per 100 cc.), chlorides appear in the urine in increasing amounts. At a concentration of about 600 mgm. per 100 cc. in the blood, the ratio $\frac{\text{Urine Cl}}{\text{Blood Cl}}$ reaches a value of 1, but it never exceeds unity by more than a very small fraction if at all. At this point the frog shows definite symptoms of poisoning. Similarly, increasing the bicarbonate in the blood produces a relatively greater concentration in the urine, but not until toxic effects are apparent does the urine value equal that of the blood. Phosphates may be concentrated 3 or 4 times by the kidney when the blood phosphate is increased. The total salt content of the urine is always less than that of the blood. With glucose or phlorhidzin injections the urine may contain 2 or 3 times as much sugar as the blood, but simultaneous determinations of urea show the latter to be much more highly concentrated, as is also the case when phosphate and urea determinations are compared. When the urea or phenol red in the blood is increased the concentration by the kidney is lessened rather than increased until the urine value approximately equals that of the blood.

The differences between the amphibian and the mammalian kidney, as observed in this work, are the inability of the amphibian kidney to concentrate chloride or bicarbonate and the much greater concentrating power for urea compared to the other substances in frog's urine.

Energy metabolism of full-term and premature infants with special reference to the influence of food and crying. M. ELIZABETH MARSH (introduced by JOHN R. MURLIN), Department of Vital Economics, University of Rochester and from the Obstetrical Division, Highland Hospital, Rochester, N. Y.

Two hundred and thirty-four observation periods were made upon fifty normal new-born infants ranging in age from six hours to fifteen days but of this number only ninety-eight periods with thirty-eight infants were truly basal, i.e., when the infant slept quietly throughout.

The basal metabolism of these thirty-eight infants averaged 6.67 calories per hour or 2.00 calories per kilogram and 29.16 calories per square meter per hour (Lissauer formula) but varied with age being the highest in the second twenty-four hours and on the basis of surface falling gradually to the sixth day from which point it rose steadily.

The respiratory quotient in these basal periods ranges from 0.66 to 1.16. The average of all quotients whether basal or not is from 0.79 the first twenty-four hours to 0.75 on the fourth and thence gradually upward to 0.85 on the ninth day.

Applying statistical methods it is found that surface area is, as usual, a slightly better measure of basal metabolism than body weight and furthermore there is practically no correlation between heat production and pulse rate.

With regard to the influence of crying on energy metabolism, the interesting observation is made that in the average infant, active, healthy cry-

ing requires just as much again expenditure of energy as the basal metabolism. Expressed differently, crying 1 per cent of the time raises the metabolism 1 per cent.

Especial attention was given to the effects of natural food and of supplementary feedings of lactose and dextrose. It was found particularly difficult to raise the respiratory quotient by means of supplementary feedings on the second and third days and likewise the dynamic action of ordinary and of supplementary feedings within the first eight days is very small. The largest recorded was 12 per cent from a feeding of 10 per cent lactose. Dextrose was about the same as lactose. Comparing the effects of small feedings (averaging 26.7 gm.) of food or of the sugar solution with that of large feedings (averaging 51 gm.) the increase in the basal heat production averaged approximately 7 per cent.

In eighty-two observation periods upon twenty-one infants whose prematurity ranged from two weeks to two months, the most striking difference was the lowered heat production, the basal metabolism being 2.91 calories lower than full-term babies when measured per square meter per hour. The influence of food and of activity was practically the same as in the full-term infants.

This study will be published in full in the American Journal of Diseases of Children.

Administration of insulin by alimentary tract. JOHN R. MURLIN, Department of Vital Economics, University of Rochester, Rochester, N. Y.

Some further favorable results looking to the successful control of diabetic symptoms by means of insulin administered in the form of a tablet have been obtained. For this purpose dogs subjected to the action of epinephrin or of ether have been used instead of animals rendered truly diabetic by the removal of the pancreas. The reason for using animals thus prepared as test subjects is that when the pancreas is removed, owing to the absence of trypsin, one of the main risks which insulin must run in the alimentary tract is absent.

Both epinephrin and ether produce, when given in sufficient amount, an alteration in the carbohydrate metabolism which simulates but does not duplicate one of the major symptoms of diabetes, namely, the high blood sugar. This hyperglycemia is caused not by failure of combustion of sugar, but by conversion of glycogen stored in the tissues, especially the liver, to glucose. However, there is a growing conviction that the function of insulin in the animal body is to produce glycogen and to prevent its too rapid conversion to sugar as well as to provide for the breakdown and combustion of sugar. Since insulin is known[1] to counteract the conversion of glycogen to sugar, in animals treated with epinephrin or placed under ether anesthesia, any evidence of such inhibition when insulin is placed in the alimentary tract of these animals may be accepted as proof of its absorption therefrom, and the proper conditions for its administration in convenient tablet form may be worked out.

More than a hundred experiments directed to this purpose have been performed. Only a few of the more successful ones can be described. Clear evidence of insulin absorption has been obtained both from acid and alkaline media in the intestine, provided the reaction is outside the range

[1] Banting, Best, Collip, Macleod and Noble: This Journal, 1922, lxii, 659.

for rapid inactivation of insulin by trypsin. Tablets have therefore been prepared with an enteric coating to resist stomach digestion, containing besides an insulin powder a certain proportion of dry acid or alkali. When such a tablet opens up in the intestine insulin is absorbed. Evidence of this has been obtained many times in animals and a few times in diabetic patients.[2] Two distinct kinds of enteric coatings have proven adaptable to the purpose. The composition of the tablet, however, must conform rather closely to certain established proportions of the ingredients. Further improvements to meet the variable conditions of the human alimentary tract are now being attempted.

Cardiac output. E. K. MARSHALL, JR., The Johns Hopkins Medical School.

The Fick principle has been used to determine the cardiac output on a series of normal unanesthetized dogs. The oxygen consumption determined by the use of a mask and Douglas bag and the oxygen contents of samples of blood drawn from the right and left ventricles have been used in calculating the minute volume of the heart. Fifty-seven determinations on four dogs extending over a period of about six months furnish the data for our conclusions.

Determinations made under resting conditions on the same day agree within 10 per cent, which is the possible error of the method. Figures obtained on the same animal but at different times may show a somewhat larger variation. The minute volume has increased slightly in one dog with increasing weight, in another dog changes up to 100 per cent have been observed, while in the other two animals the figures obtained are fairly constant. The average minute volumes per kilo are 109, 122, 154 and 154 cc. Changes in pulse rate from 63 to 160, which have occurred spontaneously, have not altered the minute volume. Pulse rates of 150 to 230 induced by the injection of atropine have not changed the output per minute in three of the dogs, while in the other animal the use of atropine has increased the minute volume about 30 per cent at one period of the experiments but has not affected it at other times. This last animal was the one in which the minute volume has varied most. Changes in the oxygen consumption of 30 per cent or more have not been accompanied by any change in the minute volume.

In a further series of experiments performed in collaboration with Tappan and Torrey the effect of changes in pulse rate on the minute volume has been investigated in anesthetized dogs. In five animals under morphine or pantopon changes in pulse rate from 54 to 180 have been without effect on the output per minute, while in six out of seven animals under pantopon-urethane anesthesia an increase in pulse rate has been accompanied by increases of from 20 to 160 per cent in the minute volume.

A segment respirometer for studying the effects of CO_2 in man. S. R. BURLAGE (by invitation) and C. J. WIGGERS, Western Reserve University Medical School.

At a meeting of the Physiological Society in 1917, Voegtlin and Wiggers demonstrated a recording spirometer built on the segment principle which is suitable for rebreathing experiments in dogs.

[2] Murlin, Sutter, Allen and Piper; Endocrinol., 1924, viii, 331.

The apparatus now demonstrated is an adaptation of this for use in similar experiments on man. The apparatus consists of a large ash can, the top of which has been somewhat extended and shaped as a large segment capsule. It has a capacity of 90 liters. Across the top which has a diameter of 40 cm. is stretched a piece of light rubber dam and to it is cemented a trapezoidal piece of cardboard, pivoting on the chord side of the circle. Changes in the volume content of the respirometer causes this membrane to move proportionately and these movements in turn are recorded on a drum by a lever attached to the trapezoidal plate.

At the bottom of the tank an outlet is provided upon which may be slipped the large bore heavy-walled rubber tubing leading to the mouthpiece. During calibration this tube is disconnected and this outlet is closed with a cork. High up at the side is a small stopcock which is kept closed while the respirometer is in use, except for the short intervals during which air samples are withdrawn and analyzed for CO_2 content. For student use the Marriott calorimetric method is sufficient although more precise determination of O_2 and CO_2 may obviously be made. This stopcock can also be connected with a Bohr meter for calibrating the respirometer, and with the air line for ventilating the tank after each experiment. After calibration, a set of abscissae writers are set on the drum and from them the tidal or minute volume can be calculated.

The subject is connected to the apparatus by a rubber tube and mouthpiece after application of the nasal clip. Every 4 minutes a 150 cc. sample of air is received into sampling tubes filled with acidulated water and a mark made on the drum. This is repeated until the CO_2 content is equal to 4 to 5 per cent. As a rule this is accomplished in 15 to 20 minutes and as the air in the tank still contains over 12 per cent volume per cent of O_2 the results are not seriously affected by anoxemia. If the experiment continues 30 minutes or more, however, the O_2 percentage falls to 10 volumes per cent or below and anoxemia effects arise. Consequently it is desirable not to continue experiment more than 20 minutes regardless of the CO_2 percentage reached.

This apparatus has been successfully employed for 2 years in the Student Physiology Laboratory of Western Reserve University and forms an excellent means of demonstrating the CO_2 control of the respiration. Other experimental uses of this apparatus naturally suggest themselves.

A photokymograph for students. CARL J. WIGGERS, Western Reserve University Medical School.

The only way to place before students a correct conception as to the nature of the arterial and venous pulse waves is to introduce the optical methods of recording into the student laboratory. On account of the greater sensitivity of the segment capsules employed, the technic of registration is much easier than with sphygmo-polygraphs. The expense incurred in equipping laboratories with segment capsules and simple illuminating systems is not great, in laboratories equipped with shop facilities they can easily be manufactured.

The chief expense arises in the purchase of duplicate photokymographs. To meet this need a less expensive form of student kymograph was devised. This consists essentially of a Harvard kymograph enclosed in a box, which has a frontal slit allowing exposure of the upper and lower portions of the paper. A test tube of water serves as a cylindrical lens. The photokymo-

graph is in fact a mechanically improved form of an apparatus described by E. L. Porter and V. W. Hart in connection with a recent investigation.[1]

Reactions to progressive anoxemia in animals with denervated heart. CHARLES W. GREENE, EUGENE PAYNE (by invitation) and ROBERT SIDDLE (by invitation), University of Missouri.

We have made comparisons of the effects of progressive asphyxiation on 1, intact animals; 2, animals with bilateral section of the vagus; 3, with similar section of the accelerator nerves, and 4, adrenalectomized animals. While we have tested twenty odd animals certain aspects of the work are still insufficiently supported to permit final decisions. Our experiments are as follows:

1. We have confirmed the type of circulatory response to progressive deprivation of oxygen outlined from previous experiences. The responses are the same when the suprarenals are first removed.

2. Animals with both vagi sectioned present no qualitative differences from the normals in the pre-crisis responses. Progressive cardiac acceleration occurs in both with greater or less increase in blood pressure. In dogs tested before and following double vagotomy the crisis heart rate is higher after vagotomy. But the acceleration may be to the same level or be even less than in the normal. If the vagal tone is pronounced, as shown at sectioning, then the initial heart rates afterward start from a higher level and will reach a higher rate at the crisis. Such observations suggest that removal of vagal tone may indeed be a factor in the acceleration of progressive anoxemia. But accelerator mechanisms are primarily responsible for the pre-crisis acceleration. Examples:

TABLE 1

Increase in the heart rate at the anoxemial crisis

		NORMAL	ANOXEMIAL CRISIS
Dog 2	Before vagotomy	136	192
	After vagotomy	160	200
Dog 4	Before vagotomy	114	144
	After vagotomy	108	192

If the suprarenals are first removed the rate responses are qualitatively the same.

TABLE 2

Heart rates after adrenalectomy

		NORMAL	ANOXEMIAL CRISIS
Dog 18	Before vagotomy	168	192
	After vagotomy	216	240

Thus far we have noted no qualitative differences before and after adrenalectomy, and no quantitative differences not explained satisfactorily by the normal range of fluctuation in such tests.

[1] E. L. Porter and V. W. Hart: This Journal, 1923, lxvi, 394.

3. After section of the vagi and all the accelerator nerves to the cardiac plexus we have failed to get the usual responses to oxygen want. The blood pressure is unchanged or falls as the asphyxial crisis is approached. The heart rates are remarkably constant throughout.

According to the views of Doctor Cannon one should find indirect cardiac acceleration from the asphyxial discharge of epinephrin. Thus far we have no evidence of such a discharge in the dog. In one exceptional case of acceleration in the denervated heart the adrenals were removed at the outset.

4. Both man and animals fail to develop the extreme vaso-constrictions and the resulting high tensions, when sufficient time is allowed for adjustments to progressive anoxemia. If men are allowed 20 to 25 minutes, and dogs 15 to 20 minutes the post asphyxial rise of the older authors seldom occurs. We believe that accumulation of CO_2 cannot occur, that adequate time is allowed for distribution of incompletely oxidized substances, and in consequence that a clearer expression of the pure effects of oxygen want is obtained by this method than by the methods of abrupt occlusion.

The effect of castration on voluntary activity. R. G. Hoskins, Ohio State University, Columbus.

Sixteen control and sixteen castrated white rats were studied by means of automatically recording revolving cages. The animals were castrated at about seventy days of age after a twenty days' preliminary study. Beginning at about the twelfth day on the average the castrated animals began to lag behind the controls. At the fiftieth day after operation the average daily activity of the control group was at its highest point, namely, 15,142 revolutions (approximately 48,000 feet) and of the castrated group, 3283 revolutions. At the end of 100 days the average records were respectively 6052 and 3172 revolutions. The highest total activity for the entire period for an individual control animal was 1,593,110 revolutions (approximately 950 miles) and for a castrated, 888,490 revolutions.

The effect of novasurol on the composition of blood and urine. Norman M. Keith and Mary Whelan, Mayo Foundation, University of Minnesota.

We studied the effects produced by novasurol on certain constituents of the blood and urine in normal human beings, the dog, and patients with ascites. Saxyl and Heilig first demonstrated the diuretic properties of novasurol in cases of cardiac decompensation with marked edema. They noted a relative and absolute increase in the excretion of chlorids in the urine during diuresis. Nonnenbruch and Muhling later proved that in normal persons novasurol caused an increased excretion of chlorid in the urine, although diuresis did not always occur. We have confirmed this finding in our normal controls and in dogs. We also noted a relative and absolute increase in the output of sodium. There was no striking change in the hydrogen-ion concentration, in the excretion of potassium, phosphates, urea, ammonia, or total nitrogen. Blood and serum analyses showed no constant changes in the concentration of hemoglobin, chlorin, sodium, potassium, calcium or phosphates. The combining power of carbon dioxid was within normal limits.

Novasurol produced definite diuresis in two patients: one with Banti's disease, the other with cirrhosis of the liver, on thirteen different occa-

sions. The constant diet was poor in salts, and low in fluid content. During these diuretic periods the urinary excretion of chlorin and sodium was relatively and absolutely increased, that of potassium variable, although usually absolutely increased. Calcium deviated little from the normal, the phosphates showed a decreased concentration, but the total output was unchanged. The total output of urea, ammonia and total nitrogen was slightly increased. The changes in blood and serum concentration during diuresis were not constant. In certain instances both an increase and a decrease in the concentration of hemoglobin or serum protein occurred in the same patient during the different diuretic periods. The chlorine concentration either remained unaltered, or a distinct decrease occurred. No constant changes were noted in the concentration of sodium, potassium, calcium or phosphates, or in the combining power of carbon dioxid.

These studies indicate that novasurol produces an increased excretion of sodium and chlorin in the urine. These two ions are usually, but not always excreted in molecular proportions. Whether this specific reaction of novasurol is due to its action on the kidneys alone, or on both the kidneys and tissues generally, has yet to be determined.

The transformation of leucocytes into macrophages, epithelioid cells, and giant cells in cultures of pure blood. WARREN H. LEWIS and MARGARET R. LEWIS, Johns Hopkins Medical School.

In hanging drops of pure blood the monocytes undergo very marked changes. Some of them exhibit high phagocytic activity, especially for the red blood-cells which they ingest and digest in large numbers. Such cells become indistinguishable from the large macrophages so abundant in the tissue spaces, the spleen, and the liver. Other monocytes not so favorably located for contact with the red cells appear to absorb ultramicroscopic material which they segregate into granules that collect about the centrosome, thus forming a central area which has a marked affinity for neutral red. Coincident with this, varying numbers of fat globules accumulate about the central area into an intermediate zone. These cells are precisely like the living epithelioid cells from the tubercles in spreads of fresh tuberculous lungs. Some of the epithelioid cells in the hanging drops hypertrophy and form multinucleated giant cells also precisely similar to those in tubercles.

Many intermediate forms between the macrophages and the epithelioid cells arise, and macrophages can change into epithelioid cells and epithelioid cells into macrophages. The monocytes, macrophages, and epithelioid cells thus appear to be merely different functional states of the same cell type. The differences in appearance of these cells are dependent, to a large extent, on the amount and character of the ingested stuffs and on the degree of digestion which they have undergone.

Thus the macrophages—the great protective phagocytic cells of the body—are probably derived from the monocytes of the blood, as are also the epithelioid cells so characteristic of tuberculous lesions.

Observations on blood lactic acid after insulin. C. H. BEST and J. H. RIDOUT, University of Toronto.

The fate of the sugar which disappears from the blood of animals under the influence of insulin is still very obscure. The interesting conclusion reached by Briggs, Koechig, Doisy and Weber that there is an increase in

blood lactic acid "parallel with" the fall in dextrose seemed worthy of further investigation. These workers state that at the time of taking the blood after the injection of insulin the animal was in a drowsy asthenic condition. Hepburn, Latchford, McCormick and Macleod have reported one experiment in which the blood sugar of an etherized dog was reduced very markedly by insulin without any significant increase in lactic acid.

Since the blood sugar of dogs may be lowered very rapidly and to very low levels by insulin without the appearance of an asthenic condition or of hyperirritability it seemed advisable to study the blood lactic acid before the appearance of these complicating factors. Accordingly we have estimated the blood lactic acid before and at varying intervals from 15 minutes to 4½ hours after insulin. In every instance the administration of insulin was followed by a marked fall in blood sugar.

In the control experiments in which saline was injected a slight decrease in blood lactic acid was usually noted. The administration of inert insulin solution produced similar results. In approximately thirty experiments in which insulin was administered a slight rise of lactic acid resulted in the great majority of instances. In many cases, however, the rise was negligible and in no instance would it account for more than a small percentage of the glucose which disappeared. Similar results were obtained with diabetic dogs and the discrepancy between the marked decrease in blood sugar and the slight rise in lactic acid is very pronounced in these experiments. In a number of experiments the concentration of hemoglobin has been followed. In many cases the fall in blood sugar was accompanied by a rise in hemoglobin concentration in both normal and diabetic animals. Hemoglobin was usually estimated before and from one to two hours after insulin. These results are confirmatory of those of Drabkin and Edwards.

Our results do not support the view that in insulin hypoglycemia, lactic acid accumulates in the blood in amounts which suggest that its formation runs "parallel with" the simultaneous disappearance of the blood dextrose.

Further observations on the analysis of the action current in nerve. JOSEPH ERLANGER, GEO. H. BISHOP and H. S. GASSER, Washington University.

By means of the cathode ray oscillograph technique it has been found that *alpha*, *beta*, *gamma* and *delta* components of the sciatic nerve of the frog enter the cord through the posterior root (VIII); that the anterior root carries *alpha* only. The sensory *alpha* process arrives in its root about 0.1 –0.2σ later than motor *alpha* in its root. The difference is due either to a slight difference in propagation rates or to the passage of sensory *alpha* to and from the posterior root ganglion. There often is in the posterior root a small *pre-alpha* action current having a slightly lower threshold and traveling a bit faster than sensory *alpha*. In the saphenous of the dog we have found, in addition to *alpha* and *beta*, a process traveling at the slow rate of 12 to 15 M.p.s. and having about the same relative threshold as *delta* in the frog. Since *gamma* is present in the sciatic and in the sensory root but not in the saphenous we venture to suggest that it has to do with muscle sense. Preliminary experiments show that sensory *alpha* in hind limb nerves evokes motor hind limb and depressor reflexes. Stimuli strong enough to start *beta* may elicit respiratory and pressor reflexes, though the latter may appear only with *delta*.

The simple action current in the phrenic nerve and the components, *alpha*, *beta*, etc., of mixed nerve broaden and diminish in height during

propagation. This, we now find, is due to differences in the rate of propagation of the constituent *axon action currents*. The evidence, briefly, is as follows: 1, Despite this change in form the energy content (area of wave and deflection of critically damped galvanometer) of the action current remains constant. 2. If a just maximal *alpha* process descending the nerve is made to pass through a submaximal ascending *alpha* process, the amplitude and time relations of the former are so altered as to indicate *a*, that the more irritable fibers are the more quickly conducting, and *b*, that the quickest traveling action currents have been blocked. 3, The absolute refractory phase of the conducted action current begins, not with the beginning of the action current, but later and by an interval that is accounted for by the conduction time of the slowest moving of the axon action currents; whereas the absolute refractory phase ends the same time interval after the beginning of the action current at all points along the nerve, this interval being, presumably, the refractory phase of the first of the component axon processes.

Some effects of experimental lesions of the mid-brain in cats. F. H. PIKE and D. A. KLENKE, Columbia University.

The relation of decerebrate rigidity to the integrity of the mid-brain and the effect of total transverse lesions of this region upon respiratory movements indicates that it has some important connection with proprioceptive fibers. There is also anatomical evidence in favor of such a view. It has seemed worth while to investigate the effects of smaller circumscribed lesions of this region upon the gait and posture of animals.

Cats were used for the experiments. Anesthesia was by ether or ether and urethane. The occipito-atlantoid ligament was exposed and incised, and the approach to the posterior corpora quadrigemina was made through the fourth ventricle. A lesion was made by inserting the point of a small curved knife into the lower end of the aqueduct of Sylvius and drawing it outward and slightly upward or, in one case, almost directly upward. In another experiment a small rounded probe was thrust into the reticular formation just beneath the opening of the aqueduct.

In one case in which, as was shown at autopsy, the incision completely severed the left inferior colliculus from the deeper lying structures, the animal was allowed to live about three months. There was severe incoordination of movement, particularly of the left side which persisted as long as the cat lived. The immediate effects were so severe as to preclude any actual locomotion whatever, but at the end of two weeks there was considerable power of locomotion. The pupils were wide and, when a strong light was thrown on them, would contract slightly and then almost immediately dilate to their former size.

About two months after the first operation, the left internal ear was removed. The cat never recovered its ability to walk about in the three weeks during which it was allowed to live subsequent to the vestibular operation. Similar results were observed in another cat in which the incision in the mid-brain had extended from the aqueduct almost directly upward. The torsion of the head was extreme in both cases. Normal cats recover the power of locomotion in three or four days after removal of an internal ear.

The dorsal part of the mid-brain must be regarded as one of the central ganglia of the proprioceptors, lesions of which are attended by severe

disturbances of locomotion. The effects of combined lesions of the inferior colliculi and vestibule show that the vestibular system plays an important rôle in the recovery from the immediate effects of the mid-brain lesion.

Data on the nature of propagation in nerve. GEO. H. BISHOP, JOSEPH ERLANGER and H. S. GASSER, Laboratories of Physiology and Pharmacology, Washington University Medical School.

1. The potential gradient from the action potential wave in nerve, extending into the unexcited region ahead, which might act as a stimulus to the nerve, resulting in propagation, has been recorded by means of the cathode ray oscillograph, in the VIIIth sensory root of the bullfrog sciatic. The potential existing ahead of the beginning of the refractory phase may be as low as 1.5 millivolts, for an action potential recorded as 30 millivolts, and rises from one-tenth of this value in not greater than 0.000,075 second, probably less. This time-potential value seems extremely small to serve as a stimulus, compared to the value of an induction shock necessary to stimulate; but the fact must be considered that the applied shock passes through many axons in series across the nerve, while the action potential is recorded from axons in parallel.

2. From the shapes of the rising and falling phases of the potential wave together with the effects on the wave of experimental conditions, the hypothesis is developed that the discharge due to excitation is the result of one reaction, possibly located in the neurofibrils, which proceeds logarithmically, until abruptly inhibited by the second or recovery reaction, possibly between fibrils and neuroplasm substance; and that the resting condition of the nerve represents an equilibrium between the two reactions. Whatever factors such as polarisation (negative), cooling, etc., lower this equilibrium value, by retarding the recovery process, allow the nerve to be stimulated at a lower threshold, and may prolong recovery, i.e., the refractory phase, with slower conduction rate. Positive polarisation, warming, etc., raise the threshold, etc., by a reversal of the equilibrium shift. The burden of regulating the nerve's condition is thrown on the recovery process, which seems to have a high temperature coefficient, the discharge reaction being more or less unaffected by extraneous circumstances. The reactions of the nerve presumably take place across a boundary or membrane, possibly between fibril and neuroplasm, and tend to polarise this boundary in opposite directions, the reaction being thus controlled by the resultant potential as at an electrode. Whatever disturbs the recovery process allows the nerve to discharge, i.e., to "become active."

Systemic response to bright light in the dog. C. I. REED, Department of Physiology, University of Chicago.

Exposure of etherized dogs to light from a 1000 wtt. tungsten bulb lantern of an intensity of 100 f. c. or to a carbon arc of 1250 f. c. intensity, which shows wave lengths longer than 3300 A° only, causes a profound depression in blood pressure. This effect results from direct exposure of the eye; direct exposure with both vagi cut; direct exposure with the optic nerve crushed; exposure of the pharyngeal mucosa; or from exposure of the blood circulated through a glass tube outside the body.

From these results it appears that blood may be affected directly by light and that visible rays may exercise a pronounced abiotic effect.

The respiratory exchange during and after muscular work under low barometric pressures. EDWARD C. SCHNEIDER and ROBERT W. CLARKE, Wesleyan University.

The consumption of oxygen is descreased during physical work by a reduction of barometric pressure. The decrease in consumption is greatest when heavy loads are carried and is roughly proportionate to the reduction in barometric pressure.

The "oxygen debt," the excess of oxygen used in the period immediately following exercise, is not materially changed by a reduction of barometric pressure. This is interpreted to mean that work done under a low barometric pressure does not cause an excessive, or unusual, production of lactic acid. Further support for this conclusion is found in the amount of carbon dioxide eliminated. Immediately after exercise some carbon dioxide is freed from the bicarbonate of the blood by lactic acid that is escaping from the muscles. The amount, and the period, of excessive elimination of carbon dioxide is, as a rule, the same at the several barometric pressures employed. After the period of excessive elimination there follows one of retention of carbon dioxide. The amount so retained also gives a measure of the amount of lactic acid formed during work. The retention of carbon dioxide is practically the same for equal loads at the various barometric pressures studied.

The response to occlusion of the head arteries after adrenalectomy. HELEN C. COOMBS, Department of Physiology, New York University and Bellevue Medical College.

In a detailed study of the effects of repeated occlusion of the head arteries in the cat, the question arose as to the possible rôle of the adrenals under the conditions of extraordinary vasomotor activity resulting from bulbar anemia. Ten to eighteen successive occlusions may be done in the normal animal.

A number of acute experiments were made in which after the control occlusion of the head arteries (blood pressure being recorded) the adrenals were excised by the extra-peritoneal method of Stewart and Rogoff. There was no immediate fall of blood pressure. From three to seven occlusions could then be obtained before pressure fell permanently to spinal level. The number of successive responses was distinctly less than in the normal animal.

After the failure of the usual cardio-vascular response to bulbar anemia in animals whose adrenals have been removed, the intravenous injection of a commercial preparation of adrenalin would so restore conditions that a typical rise of blood pressure could again be obtained on occlusion of the head arteries. Sometimes a second or even a third injection of adrenalin could be made with the subsequent restoration of vasomotor function. Injection of adrenalin does not restore conditions in the normal cat after failure of the vascular response to occlusion of the head arteries.

There were a few animals—young cats—in which twelve to fifteen successive occlusions were followed by the usual vasomotor response. Two were control occlusions and the others were done after adrenalectomy. Intravenous injection of adrenalin so restored conditions that further responses to occlusion of the head arteries were obtained. In these animals, however, there was a strong vagus effect present from the beginning, so that the blood pressure never rose very high during the occlusions. It

has previously been shown[1] that the vagus exerts a sparing or conservative action upon the whole mechanism concerned in the response to bulbar anemia

Two conclusions seem justified at present. Some product of the adrenals —whether medullary or cortical is still in doubt—is necessary for the continued action of sympathetic nerve upon the smooth muscle of the vascular walls. Although there may be an increase in the rate of liberation of epinephrin during cerebral anemia in the intact animal, the fact that the usual type of vasomotor response follows after excision of the adrenals[2] shows that no actual increase either in the rate of liberation or the concentration in the blood is necessary for the excitatory action of sympathetic nerve upon the smooth muscle of the blood vessels.

Theoretically, one might expect from Elliott's demonstration[3] that epinephrin has the same effect upon any structure or organ as the sympathetic nerve to that structure or organ, that epinephrin might be concerned in the process of stimulation of smooth muscle or gland. If these structures could be set into activity, one might expect that epinephrin might be consumed more rapidly than usual and that this high state of activity would lead to eventual failure. This appears to be what happens under the conditions of increased vasomotor activity elicited by bulbar anemia.

Further observations on experimental aortic regurgitation. H. C. Bazett, Department of Physiology, University of Pennsylvania Medical School, Philadelphia.

A brief report of some of our work on experimental aortic regurgitation in dogs has already been made.[4] Further work indicates that considerable hypertrophy may eventually occur but usually develops slowly. In such cases the Q R S group becomes of longer duration, and the Q and R waves show a considerable increase in potential in all leads, while the S wave is diminished or eliminated.

The hypertrophy is accompanied by a considerable increase in the capacity of the left ventricle, in comparison with that of the right. Thus in one case the capacity of the left ventricle was 27 cc. that of the right 19.5 cc. only. In the early stages following operation the diastolic murmur is recordable throughout diastole. Later, after the development of hypertrophy, the murmur is less definite in the latter half of diastole.

While at first sight the immediate diminution in heart size following the production of the lesion, previously reported, might appear to support H. A. Stewart's theory[5] of a regurgitation of pressure with minimal regurgitation of fluid into the ventricle, this hypothesis would not account for murmurs of long duration, for the increased capacity of the left ventricle in long standing cases, for an actual lengthening of the heart shadow often seen after operation even though the total area is not increased, nor for experimental results such as one where the pulse rate rose following the operation from 79 to 133 yet without any diminution in the heart size.

The effects of the operation on the heart size can only be understood if

[1] Coombs, H. C.: This Journal, 1924, lxviii, 124.
[2] Rogoff, J. M.: This Journal, 1924, lxvii, 551.
[3] Elliott, T. R.: Journ. Physiol., 1905, xxxii, 401.
[4] Sands: Quart. Journ. Exper. Physiol., 1923. Supplementary vol. p. 215.
[5] Stewart: Arch. Int. Med., 1908, 1, 102.

one remembers that the shadow of the left ventricle is only a portion of the total shadow (about 44 per cent in the antero-posterior photographs) and that if the pulse rate is doubled, as it commonly is, a reduction in size in the auricles and right ventricle must be expected. If the heart shadow remains unchanged in spite of the increased pulse rate, it indicates an increase in the left ventricle equivalent to the decrease in the other chambers or else a considerable loss of tone in the whole heart. There is no reason to suppose the latter.

Stewart's theory was founded on plethysmographic records, in which the condition of the left ventricle was assumed from records showing the volume changes of both ventricles together, regardless of the fact that there was no probability that the right ventricular output remained uninfluenced by the operation.

The experimental results here reported agree very well with those of Stewart, but not at all with the conclusions he drew, since the actual lengthening of a heart of total diminished volume is best explained on an unequal filling of the two ventricles.

On the surface composition of normal and sensitized blood cells. STUART MUDD and EMILY B. H. MUDD, Laboratories of the Rockefeller Institute for Medical Research.

We have described a method for determining certain interfacial tension relations between bacterial or other cells and two liquid phases. The cells are viewed in a film at the boundary between aqueous and organic phases with a darkfield microscope. From the relations thus discerned, inferences as to the chemical categories of the substances in the cell surfaces may be drawn.[1]

The cells of human, horse, donkey and sheep blood have been thus studied in films against a variety of oils. Red and white cells differ sharply in response. The erythrocytes on touching the interface are readily wet by the oils, passing with the aid of very little mechanical work into the organic phase. This rule has applied whether the cells are normal, crenated, or "ghosts," and whether suspended in dilute plasma or serum, in "physiological" NaCl, or in Ringer-Locke solution. Sheep cells are exceptional in that they have been markedly stable in the interfaces of certain preparations, requiring considerable work to drag them into the oil. Freely suspended red cells while in the interface are pulled into a characteristic lens shape with the long axes in the plane of the liquid-liquid tension.

Leucocytes, on the other hand, are not readily wet by the oils. If suspended they are ordinarily pushed before the advancing oil for a time. They may press back the interface until it forms a peninsula of water which pinches off, leaving the leucocyte enclosed in a vacuole of water in the oil. Or the leucocyte may spread in the interface, completely disintegrating.

Red cells, when strongly sensitized with anti-erythrocyte serum, are no longer easily wet by the oils, but are stable in the oil-aqueous phase interface. If freely suspended they slide along the interface; if stuck to the glass and encroached on by the oil phase they push the interface backward; if dragged through into the oil they are often pulled out into pear or dumbbell shapes and may even pinch in two. The changed reaction of specifically

[1] Mudd, S. and E. B. H. Mudd: Journ. Exper. Med., 1924, xl, 633, 647.

sensitized erythrocytes has been detected with a specimen of anti-human erythrocyte serum in dilutions up to 0.3 per cent serum containing 1 per cent of human cells; with specimens of anti-horse serum the reaction is detectable in dilutions up to 2 per cent serum and 1 per cent horse cells. Anti-human serum is ineffective with horse cells.

The following conclusions are drawn from these and other data. The surfaces of normal erythrocytes of the species studied contain in large amount but not exclusively non-polar, presumably lipoidal substances. Specific sensitizing serum alters this non-polar portion of the erythrocyte surface in such a way as to make it more polar. The surfaces of leucocytes of the type which bend back the interface at least, are predominantly polar.

The causes of gastric secretion with a consideration of the mechanism concerned. A. C. Ivy, R. K. S. Lim, J. B. McCarthy and J. I. Farrell, Hull Physiological Laboratory, The University of Chicago.

The observations of Ivy, McIlvain and Javois on the stimulation of the gastric glands by the action of the digestive products of foods in or via the intestine have been confirmed by preparing dogs with a pouch of the entire stomach and a duodeno-esophageal anastomosis. Such an animal following the ingestion of a meal usually secretes from 150 to 300 cc. of gastric juice in 24 hours, the secretion beginning from 1 to 3 hours after the meal is ingested.

Using this preparation, we have found that mechanical distention of the pouch of the entire stomach (vagi cut) with a balloon (200 to 250 cc.) stimulates the gastric glands; also, that meat juice (20 to 30) cc. is the only one of the common food substances that stimulates gastric secretion by local action in the stomach. Histamine (2 cc. of 1:1000) and β-alanine (20 cc. water containing 200 mgm.) have an action similar to meat juice.

It is now necessary to divide gastric secretion into three phases as related to cause. 1, The cephalic phase caused by *a*, reflexes through the cerebral cortex (psychic), and by *b*, reflexes through the thalamus, mid-brain and medulla. 2, The gastric phase caused by *a*, mechanical distention of the stomach and by *b*, "chemical" action on or via the gastric mucosa of certain substances. 3, The intestinal phase caused by "chemical" action on or via the intestine of certain substances.

In order to ascertain the mechanism concerned in the second and third phases, we have performed a series of blood transfusions and four long-time (3 to 4 days) cross-circulation experiments, using Pavlov pouch dogs. From 150 to 200 cc. of compatible blood was transfused by the whole blood (Percy tube) method. A few positive results occurred when both "starved" and "fed" blood was transfused; hence, we concluded that such an experiment was inadequate for our purpose. The cross-circulation experiments were performed by making an end-to-end anastomosis of carotid-to-carotid or carotid-to-carotid and external jugular-to-external jugular. In the former experiments it was necessary to transplant a piece of carotid (3 in.) from a third dog. The skin wounds were closed and the two animals were put in a plaster cast and made comfortable in a hammock. They were fed alternately at long intervals (12 hours) to see if the ingestion of a meal by one would increase the gastric secretion of both. The cross-circulation was proven by an injection of methylene blue into one animal and

its recovery in the gastric juice of both. Both animals were in good condition as shown by a normal gastric secretory response to a meal. Out of some ten tests in the four experiments only one suggestive, but inconclusive, response occurred, which could possibly be accounted for by a normal variation in the continuous secretion.

Recently two of us (Ivy and Farrell) by a two stage operation have been successful in transplanting a small pouch of the stomach (fundus) into the mammary gland of two dogs that had just previously weaned their pups. One of these animals has given positive and unquestionable evidence of stimulation of gastric secretion from three to four hours after the ingestion of a meal. The second animal, although the "take" is good, had shown only an increase in total acidity and quantity after a meal. We tentatively conclude that this is unquestionable proof that a humoral mechanism is concerned in gastric secretion.

The basal metabolism of normal immature white rats. ADDISON GULICK, University of Missouri.

Tests were made by gravimetric CO_2 method on sleeping rats (mostly males) at 29.5° — 31°C., 18 hours or more after last replenishing their food. Results were compared through I, the metabolic index number: $I = \frac{Cal.}{F}$, in which the length-weight factor, $F = W^{.425} \times L^{.725}$. The units are kilograms, centimeters from nose to anus, and great calories per hour. $F = 12.8 \times W^{\frac{2}{3}}$ in well-nourished rats 4 to 9 weeks old, but not in poorly nourished, nor in adults.

In the first 5 weeks gas exchange is in nearly direct proportion to weight, each increasing more than 10 fold, thus causing the value of I to be doubled in the course of the 5 weeks. During the 6th week (75 gm. at start, growing to 111 gm.) I is stationary at its maximum value, averaging 0.303, while $Cal. \div W^{\frac{2}{3}}$ averages 3.81. After that I falls somewhat, to approach adult normal.

The two physiological mechanisms for blood clotting. C. A. MILLS and A. P. MATHEWS, University of Cincinnati.

The following new facts concerning blood clotting are presented:

First, tissue fibrinogen, although containing over 40 per cent cephalin, is entirely incapable of changing serozyme to thrombin in serum. Its cephalin, if liberated from the union with the protein, is just as efficient an activator as cephalin from any other source. Serum from tissue fibrinogen clotting has its serozyme content left intact. That from cephalin clotting has its serozyme completely exhausted.

Second, Bordet's phosphated plasma, entirely freed of serozyme and platelets and incapable of clotting with calcium alone, or with cephalin and calcium, clots fully as well as the normal plasma on addition of tissue fibrinogen and calcium. Clotting of this plasma with tissue fibrinogen yields no trace of thrombin in the serum.

Third, intravascular clotting and death in a few seconds may be produced by thrombin just as surely as with active tissue extracts. Serum rich in serozyme need only be activated by cephalin and injected intravenously within two to three minutes to produce such results. If left standing thirty minutes after activation, no symptoms follow its injection in any amount.

The above facts, taken together with our past work, compel us to accept both tissue fibrinogen and thrombin clotting as truly physiological, but entirely independent processes.

Further studies on parathyroid tetany. LESTER R. DRAGSTEDT, Division of Physiology and Pharmacology of Northwestern University Medical School.

The prevention and control of parathyroid tetany in dogs by the administration of strontium salts. Complete thyro-parathyroidectomy was done in 9 adult dogs. These dogs were given bread and water ad libitum after the operation. Fifteen grams of strontium lactate dissolved in water or incorporated in cornmeal mush was administered daily by a stomach tube. Seven of these dogs died in tetany at the end of 4 days, 7 days, 8 days, 11 days, 17 days, 18 days and 23 days respectively. Two recovered from tetany and are still living, eight months since the operation. Violent tetany developed a number of times in these animals and it was relieved completely by the intravenous injection of from 800 to 1200 cc. of a modified Ringer's solution in which strontium chloride was substituted in place of the calcum chloride of the usual formula. It was quite evident that strontium was not so effective as calcium in the treatment of parathyroid tetany. Strontium lactate when given by mouth appears to be quite irritant to the gastro-intestinal mucous membrane often causing vomiting and bloody stools.

The prevention and control of parathyroid tetany in dogs by the oral administration of kaolin. Complete thyro-parathyroidectomy was done in 5 adult dogs. These dogs were given bread and water ad libitum after the operation. From 50 to 150 grams of kaolin and 50 cc. of liquid petrolatum incorporated in cornmeal mush were given daily by stomach tube. Three of these dogs died in tetany after 4 days, 8 days, and 15 days respectively. Two of the dogs recovered from tetany and are still living, 2 months and 8 months since the operation. Kaolin contains approximately 47 per cent silica, 40 per cent aluminum oxide, and 13 per cent water. It is insoluble in water, cold dilute acids or alkalies. It seems most probable that its effect in these experiments is purely local, and that it prevents tetany partly because it checks bacterial proteolysis in the intestines and partly through its property of combining with toxins and toxic products of bacterial growth.

Thyroid administration and tetany. Three adult female dogs, which had survived complete thyro-parathyroidectomy for one to two years and which were known to develope tetany at oestrus and at excessive meat feeding were given desiccated thyroid daily for two weeks. Two of the dogs were given daily $\frac{1}{3}$ gram of desiccated thyroid per kilo of body weight, which amount according to Doctor Kunde will uniformly raise the basal metabolic rate of dogs 30 to 40 per cent after 4 or 5 days. Neither of these dogs developed tetany. The third dog was given 1 gram of desiccated thyroid per kilo of body weight, a total of 6.3 grams of thyroid daily for two weeks, and did not develop tetany. It is quite evident that excessive thyroid feeding does not induce in these animals the same favorable conditions for the development of tetany as accompanies oestrus, pregnancy, or lactation. It is accordingly not probable that the toxins which induce tetany under these conditions are of endogenous origin.

Diabetes mellitus in a dog with a diminutive pancreas. S. A. MATTHEWS, Department of Physiology, Therapeutics and Pharmacology, Loyola University School of Medicine, Chicago.

The material for this report was obtained from an apparently healthy female dog, weight 11 kgm. During an operation on this animal which involved the pancreas what seemed to be a total absence of the pancreas was discovered. Immediately a small quantity of urine was obtained from the bladder and a specimen of blood (25 cc.) was taken from the heart. Sugar was present in the urine, and the blood showed a sugar content of 840 mgm. per 100 cc.

More careful examination revealed a nodule of tissue about 2 cm. by 1 cm. located in the mesentery about 3 cm. below the duodenum. This nodule apparently was unattached to the duodenum. Microscopic examination showed this to be pancreatic tissue, but without a sign of island tissue. Also microscopic sections of the dodenum showed no pancreatic tissue. The liver had undergone fatty degeneration to almost complete destruction. While no direct tests were made for glycogen, the liver after autolyzing for two hours showed very little sugar, which might be taken as evidence of glycogen deficiency.

Determination of the circulation rate in man by inhalation of ethyl iodide. YANDELL HENDERSON and HOWARD W. HAGGARD, Yale University.

Ever since Harvey showed that the blood circulates, the determination of the volume of flow per minute has remained the outstanding unsolved problem of the circulation. The unsatisfactory condition of knowledge on this matter, and the importance of a simple, and fairly accurate method for measuring the circulation in man, has been pointed out by Y. Henderson in Physiological Reviews.[1] The method based on absorption of nitrous oxide from the lungs (Krogh and Lindhard) is extremely inaccurate and unreliable. The application the Fick principle (the volume of oxygen absorbed from the lungs divided by the difference in the oxygen content of the arterial and venous blood) is too elaborate for use except in research. A simpler method has been sought for twenty years past in this laboratory along a wide variety of lines, and always in the end unsuccessfully.

Investigations in this laboratory during recent years have led to the formulation of the principles controlling the absorption of any gas whatever by mere solution from the lung air into the blood.[2] These principles show that the rate of absorption of a very soluble gas (e.g., ether or alcohol vapor) is dependent mainly on the minute volume of breathing, while that of a relatively slightly soluble gas is more nearly proportional to the blood stream. Gases of about the proper solubility were therefore tried, one after another, until the vapor of ethyl iodide was tested. With this substance an effective solution of the problem seems to be possible and the development of a simple and fairly accurate method is probably only a matter of details of procedure.

The advantages of ethyl iodide vapor are as follows: 1, Minute amounts of this substance, when vaporized in air are analyzable very accurately by the iodine pentoxide method; and very low concentrations of the vapor may be used. 2, The distribution coefficient of the vapor between air and blood

[1] Henderson, Y.: Physiol. Rev., 1923, iii, 165.
[2] Haggard, H. W.: Journ. Biol. Chem., 1924, lix, 753.

at body temperature is a conveniently low value, approximately 2.1. 3, The substance is not decomposed in the blood, but is decomposed so nearly completely in the tissues that the content of the venous blood returning to the lungs may be taken constantly as approximately zero, or at most as only about 5 per cent of that in the arterial blood. Thus when air to which the attenuated vapor of ethyl iodide has been added is inhaled for a considerable period, 20 minutes or more, and the expired air is sampled from time to time during this period, these samples are found to have a uniform concentration; showing that no accumulation in the body occurs. During rest the expired concentration is about half the inspired. 4, No coöperation on the part of the subject is necessary, other than to breathe as naturally as possible through a mouthpiece and valves. A preliminary period of two minutes is allowed for the concentration in the lungs to become uniform, and then the expired air is collected for 3 minutes.

The factors determined are: 1, the volume of air breathed per minute, 2, the content of ethyl iodide in the inspired air, 3, the content in the expired air, and 4, the concentration in the alveolar air. The first factor is multiplied by the difference between the second and third and gives a, the amount of ethyl iodide absorbed per minute; the fourth factor is multiplied by the coefficient of solubility and this gives b, the amount in the arterial blood; and a divided by b gives the volume of the blood flow through the lungs. Improvements in the procedures for obtaining alveolar air or estimating its composition have also been effected.

The relation of the urine reaction to the acidity of the gastric juice in Pavlov pouch dogs. T. E. Boyd and W. C. Austin, Departments of Physiology and Physiological Chemistry, Loyola University School of Medicine.

Female dogs with Pavlov pouches were used. It was felt that the curve of gastric secretion could be followed more accurately in these animals than is possible in human subjects. Moreover, the "alkaline tide," assuming that it normally occurs, might possibly be expected to appear in a more marked form, on account of the loss of some hydrochloric acid throgh the fistula. The bladder was emptied at the beginning of each experiment, and at hourly intervals thereafter. The pH was measured by the potentiometer immediately after the collection of each sample. Gastric juice was collected simultaneously with the urine. The acidity was usually determined by the potentiometer method, in a few experiments by titration.

The duration of each experiment was five to seven hours. The first hour was used for a control, after which the dog received a standard meal of bread and milk. The subsequent changes in the reaction of the gastric juice were not found to have any constant relation to the reaction of the corresponding samples of urine. In individual experiments the "alkaline tide" was present, but the urine varied toward acidity nearly as often. The composite curve of urine reaction for all the feeding experiments is a line almost horizontal.

In another series of experiments, the gastric secretion was stimulated by means of gastrin injected subcutaneously. This was done in order to eliminate the possible effect of absorbed substance on the urine reaction. There was no evidence of an alkaline tide under these conditions. Most often the maximum acidity developed in the urine and in the gastric juice at the same time. This was true even when a sustained secretion was kept up by successive injections of gastrin.

The duration of the systole of the left ventricle. WARREN P. LOMBARD and OTIS M. COPE, University of Michigan.

Using the time between the beginning of the primary wave and the bottom of the dicrotic notch of the carotid pulse curve as a measure of the systole of the left ventricle, we determined this interval in 176 men and 68 women in the standing, sitting, and recumbent positions, more than 10,000 cycles being studied. The average duration of the systoles in different cycle lengths was determined by plotting curves, with cycle lengths as abscissae and systole lengths as ordinates, and it was found that in cycle lengths 12,000–0.500 sec. the systoles decreased in a straight line as the cycles shortened, the height and pitch of the curves differing with the sex and the position. The following formulae correspond to the curves, S being systole, and C cycle.

Men standing	$S = 0.150\ C + 0.1242$
sitting	$S = 0.147\ C + 0.1478$
lying	$S = 0.105\ C + 0.2010$
Women standing	$S = 0.157\ C + 0.1375$
sitting	$S = 0.135\ C + 0.1683$
lying	$S = 0.065\ C + 0.2478$

The systole of the normal adult, determined by the average of 15 consecutive cycles, should not differ from the standard more than 0.025 second. This permissible deviation is due in part to errors of method, but chiefly to the quantity of blood which the heart has to pump. The systoles of both men and women shorten as cycles shorten, because there is less time for venous blood to accumulate, and the blood is pumped out of the veno-auricular reservoir faster. The systoles of both are shorter in the standing than the sitting, and in the sitting than the recumbent position, in all cycle lengths, because of the effects of gravity to retard the flow of the venous blood. The systoles of women are longer in like cycle lengths, in all positions, than those of men, not because the hearts of women are weaker, but probably because they are smaller in relation to the amount of blood to be pumped. The pulse of women does not change as much as that of men by change of position, as is shown by the following table.

	STANDING	SITTING	LYING DOWN
Men	81.92	73.57	63.47
Women	86.31	80.24	74.47
	4.39	6.67	11.00

The difference in the pulse rate is probably a compensatory adjustment, to lessen the amount of blood to be pumped per beat by the smaller hearts of women, the difference in the rate being the greater the larger the amount of blood that would have to be pumped.

As cycles shorten by a constant amount, systoles shorten by a constant but less amount, and occupy an ever increasing proportion of the cycles. The decrease in the duration of the diastoles is largely determined by the rate of discharge of impulses from the sino-auricular node, while the shortening of the systoles is due to the lessening of the venous supply to the heart caused by the shortening cycles. The length of the systoles does not vary with the length of the diastoles, because there is always sufficient time

for the ventricle to fill by ordinary heart rates. Age, height, weight, arterial pressure, smoking, were not found to influence the length of the systole. Henderson's "Law of uniform behavior" does not apply to the human heart.

A comparative study of equilibrium in pigeon on the ablation of cerebellum and severance of semicircular canals. G. A. TALBERT and F. L. JENKINS, (by invitation), University of North Dakota.

In the semicircular canal operations we have noted in several of the pigeons the same phenomena that have been recorded by others. However, we have seen in a few of them some remarkable recoveries.

In one instance where the lateral canal was severed on the right side, the bird was able to stand within an hour after the operation, but when he attempted to walk he staggered toward the left. Eighteen hours later it was difficult to observe any defects and even then it was only when the animal was hurried. On the third day the bird seemed to be able to walk and fly as well as the controls and was quite as able to feed himself and ward off the attacks of his cage-mates.

In another instance where all of the canals were severed on the same side, there were manifested the usual disturbances during the first three or four days which have been observed by others. However, after that there was quite a sudden improvement in walking as well as in standing. At times there would be the twisting of the neck at an angle of 180 degrees which would cause the same disturbances in equilibrium that had been observed during the first few days. At the end of seven weeks, the bird stood and walked in a very normal manner. However, he could not fly, being able to sustain himself in the air but momentarily.

In one pigeon with cerebellar extirpation, there was shown quite a remarkable readaptation after recovery from the operation. On the first day the bird rested back on his haunches and tail feathers and for several hours kept up a more or less incessant flapping of his wings. On the second day the flapping of the wings ceased. On the third day he fed himself with difficulty. On the fourth and fifth days he was better able to feed himself. On the sixth day he was seen preening his feathers and he made an attempt to fly. On the eighth day he made an attempt to stand on his feet. On the ninth day he was able to stand momentarily and he made some attempts to walk. On the tenth day he flew about four feet. On the thirteenth day he flew about thirty feet, but missed his landing. After two weeks the bird flew in quite a normal manner, and, finding his way back to his cage, landed normally. After seven weeks the bird walked with a very unsteady gait, but flew well.

In our comparisons we have noted so far that our cerebellar operated animals have manifested a more lasting disturbance in walking rather than in flying. On the other hand, the semicircular canal operations have wrought greater havoc in flying.

The effect of defective nutrition upon the behavior of the rat in a maze. ARTHUR H. SMITH and JOHN E. ANDERSON, Yale University.

A group of female rats 32 days of age was given thirty trials in a maze. On the basis of their performance they were then separated into three groups. Group I served as the control and was given ad lib a food complete in all the known dietary constituents. Group II was fed ad lib a diet

similar to that given group I with the sole exception that gliadin provided the source of nitrogen. Group III was fed the same ration as that given group I except that these rats received only sufficient food to maintain a constant weight. Group II was stunted qualitatively; group III, quantitatively, both at about the same weight level.

After an interval of 28 days each of the three groups was given eighteen trials on the original maze, twelve trials on a different maze of approximately equal difficulty and finally twelve trials on the original maze. The curves obtained when time is plotted against number of trials show that both groups of stunted rats were superior to the control group in relearning the original maze and in learning the new maze. On the other hand the curves obtained when number of errors is plotted against number of trials show that the stunted rats were superior to the control rats in relearning the old maze but inferior in learning the new maze. It is possible to differentiate between the quantitatively stunted and the qualitatively stunted rats by means of their behavior, in that the former make more errors than do the latter.

The two stunted groups were then fed the same diet ad lib as was provided for the control group and grew at a definitely accelerated rate. After 28 days each of the three groups were given twelve trials on the original maze, twelve trials on the interfering maze and finally six trials on the original maze. After realimentation both the time curves and the error curves show less discrepancy between groups than do those obtained immediately following the stunting.

The above results indicate that the normal functions of the organs concerned with the process of habit formation are interfered with by the same alterations in nutritive conditions as have been found to bring about the well known disturbances in metabolism, growth and reproduction.

Micro-dissection and injection studies on the antagonistic action of salts upon protoplasm. ROBERT CHAMBERS, Cornell University Medical College.

If the surface of a sea-urchin egg be torn in a solution of $CaCl_2$ the protoplasm of the egg immediately sets into a jelly. In $MgCl_2$ the same reaction takes place but more slowly. In NaCl and KCl, on the other hand, tearing of the protoplasmic surface film results in a dissipation of the egg protoplasm.

Corresponding results occur when the eggs are injected with the various salt solutions. $CaCl_2$ and $MgCl_2$ coagulate while NaCl and KCl liquefy the protoplasm. By using a proper combination of the salts in question a solution can be obtained which will neither coagulate nor liquefy the protoplasm. Injections of such a solution do not affect the viability of the egg.

Body temperature changes in turtles and their physiological interpretations (Chrysemys marginata belli) (Gray) (Chelydra serpentina) (Linn.) FRANCIS MARSH BALDWIN, Iowa State College.

In a series of some forty experiments on individuals of the common painted and snapping turtles of different sizes, ranging in weights from 300 to 1600 grams, the rectal fluctuations were followed through ranges of noncritical and high and low critical temperatures. In both forms a lag of from 3 to 6 degrees is recorded as the temperature gradually fluctuates

within narrow limits in the non-critical ranges above and below 60°F. Quite constant differences are noted in the rectal readings when the rate of cooling is varied. A rapid drop from room temperature to that of melting ice and there maintained for several hours (3 to 6) is accompanied by a gradual body drop. An abrupt check in the drop is noted at between 45 and 40 degrees and thereafter it is maintained at practically this level. When the environmental drop is made slowly over a period of two hours or more and then maintained at the ice level, the body drop is gradual but more rapid than in the former condition. Here as before a decided check in drop comes at about 40 degrees, and although some individual differences were noted as to the rate in drop, in no case did the body temperature approximate that of its environent, under the conditions of the experiment. Accompanying these temperature changes are noted differences in physiological activities, with muscular action at the outset which gradually merges into a period of comparative quiet and this in turn is followed at the critical temperatures noted by an interval of continuous, though slow active movements. These latter movements no doubt liberate sufficient heat to keep the body temperature from dropping at this stage.

Increase in environmental temperature is accompanied by a corresponding rise in body temperature and as a rule this is fatal if maintained at 102 to 105°F., for any considerable time (30 minutes or more). At 80 degrees and above animals show marked increased activity; signs of discomfort with rapid respiration; a frothing about the mouth and an accumulation of moisture upon the head and about the eyes.

In the absence of concrete data on comparative metabolic rate at different temperatures, these facts are tentatively interpreted to mean that there is in turtles a slight tendency to compensate for critical temperature changes in their environment.

The protosensory mechanism of pecten irradians—Preliminary note. H. K. Hartline, Marine Biological Laboratory, Woods Hole, Mass.

The photosensory mechanism in the clam, *Mya arenaria*, has been analysed by Hecht. *Mya* responds reflexly to an increase of illumination upon it. The present experiments deal with an animal which reacts to a decrease in illumination—*Pecten irradians*.

The animal is illuminated by lights of known relative intensities. On cutting off the light, the animal is stimulated and reacts by closing its valves. The instants of stimulation and response are recorded along with a tuning fork record on a revolving smoked drum. Under the same conditions, the reaction times measured in this way are consistent and reproduceable.

The reaction time varies between 0.2 and 0.4 second; it is shorter with higher intensities and at higher temperatures. Size of retinal image and depth of shadow also affects the reaction time. Keeping all other conditions constant and measuring the time of reaction to complete shading, results were obtained which can probably be explained in terms of a system such as that described by Hecht.

During illumination at intensity I, the reversible system $S \rightleftharpoons P + A$ is in the stationary state defined by the equation $KI = \frac{x^2}{a - x}$, x being the concentration of P and A; $a - x$, that of S. On darkening, the reaction goes in the direction $P + A$ S, with the velocity $v = k\,x^2$. Assuming

that a constant, small amount of P or A must be removed to produce a response, the reflex velocity becomes a measure of the velocity of chemical change, and therefore of x^2; provided that from it be subtracted the minimum velocity (obtained graphically), where $x = 0$. The maximum velocity is obtained by electrical stimulation of the mantle fringe, and gives the value of a^2, after subtracting the minimal velocity. Substituting these values as obtained from the reaction times in the above equation, a very good linear relation is obtained between log I and log $\frac{x^2}{a-x}$, indicating that Hecht's equation of the stationary state defines the conditions in the eye of *pecten*.

Some very different material, when subjected to similar treatment, behaves in the same way: recent data of Chaffee and Hampson on the magnitude of the electrical response of the frog's retina to monochromatic light of various intensity will give a good linear plot of log I against log $\frac{x^2}{a-x}$ if the response R is put equal to x, a being given a value slightly higher than the greatest response. The significance of this will be discussed elsewhere.

A difficulty arises in that the slopes of these logarithmic plots are not unity. The presence of exponents in the equations indicates a more complicated situation— as does also some preliminary experiments on the reactions of *pecten* to very brief shadings. As yet there is no explanation of the difficulty to be offered.

Influence of CO_2 retention on skeletal muscle cramp. G. W. Fitz, Peconic, Long Island, N. Y.

Author experimented on self to take advantage of idiopathic cramps to which he was subject. These occurred in thigh extensors, chiefly v. internus and v. rectus. The attempt was made to find a physiological cure for cramp, on the basis of the alkalosis theory. The conditions were unfavorable for chemical tests, as the cramps occurred unexpectedly during sleep. The results obtained were subsequently confirmed in other subjects.

Various methods of reducing the alkalinity of the blood by CO_2 accumulation were tried, as holding the breath, retarding the breathing, and re-breathing with head under the bed clothes and also from an air-tight bag.

It was found that the cramps were favorably affected in proportion to the completeness of the re-breathing. When re-breathing was made fully effective by the use of a three quart rubber cloth bag securely held over mouth and nose, the pain was immediately greatly reduced, and the cramp quickly faded out. This result has been so uniform and prompt in several subjects and many attacks involving different muscles that there can be no question that reduction in blood alkalinity by CO_2 accumulation, constitutes a true physiological cure for cramp of skeletal muscles, thus clinically confirming the alkalosis theory of cramp (tetanus) and at the same time establishing a quick practical cure for an extremely painful and at times even terrifying condition.

The author believes that idiopathic cramp of skeletal muscles is due to a critical trigger state of the muscle fibres, possibly because of an alkalosis

due to over-breathing, and that, as the alkalosis is reduced by CO_2 accumulation, this critical state is exchanged for the normal condition of obedience to nerve control for contraction and relaxation.

Irritability and blood sugar. E. M. GREISHEIMER, Department of Physiology, University of Minnesota.

Several years ago it was observed that frogs' nerves lost their irritability and conductivity fairly rapidly when immersed in cane sugar solutions. About a year ago these observations were recalled by reports of beneficial results obtained after injection of glucose in convulsions and by reports of increased irritability in patients after insulin had been given. The question of a possible relation between the irritability of the nervous system and the blood sugar level was considered.

A series of experiments was performed using decerebrate dogs. No food had been given for twenty-four hours before the experiment and ether was given just long enough for the carotid to be prepared for blood pressure tracings and for decerebration. The brachial plexus was prepared and one of its trunks severed (usually the radial). The femoral nerve was severed. The femoral artery was prepared for removal of blood samples and the femoral vein for injection.

The irritability of the nerves was determined by finding the weakest break shock capable of giving a response in the foot. The central end of the radial as well as of the femoral, gave information regarding the condition of the reflex arc. The irritability was determined at fifteen minute intervals, samples of blood for sugar determinations were taken every half hour, blood pressure records were taken throughout, and occasionally determinations of oxygen and carbon dioxide of the blood were made. The rectal temperature was recorded frequently.

Control experiments were run in which no injections were given. It was found that the irritability of the nerves remained fairly constant even over twelve to fifteen hour periods. When the blood sugar fell, the irritability increased. When the blood sugar rose, the irritability decreased. This relationship holds in both the peripheral nerves and the reflex arcs.

In several experiments, insulin was used. One hundred units were given intravenously. As the blood sugar decreased, there was usually an increase in the irritability. The exceptions suggest another relationship which has not yet been found. Curves showing the results have been plotted.

Control of the heart rate during acapnia and asphyxia. ALEITA HOPPING, Columbia University Medical School.

Two series of rabbits and one series of cats were anesthetized and the heart rate and blood pressure determined by the Hürthle manometer. After pneumothorax, overventilation of the lungs was maintained for several minutes, the air was then cut off and tracings made until the animal passed into asphyxial convulsions. Heart rates were recorded after dividing the right and left sympathetic chain proximal to the stellate ganglion and after sectioning each vagus.

Sectioning the accelerators in the rabbit is accompanied by a decreased rate during both overventilation and asphyxia. Cutting the vagi after the accelerators have been divided is followed by a decreased rate during overventilation and an increased rate during asphyxia so that in the dener-

vated heart change in rate is reducedto about one-third of that in the intact heart. Whether the increased rate in the denervated heart during asphyxia is due to adrenalin secreted during this period is being studied.

Changes in the heart rate that occur during acapnia and asphyxia are partially under control of the central nervous system, and are partly due to direct action on the heart.

Some effects of the continued injection of insulin in rabbits. HARVEY S. THATCHER and ERNEST L. SCOTT, Columbia University Medical School.

The insulin was injected subcutaneously in doses of 0.7 "U" unit per kilogram of body weight. The injections were made daily from the first of December, 1923, to the first of April, 1924. Blood sugar was determined 45 minutes after the injection at least four times a week, and the weekly averages compared with those of a control series.

No change in sensitivity which could be attributed to the insulin was noticed. However during the last week of December and the first week of January the control series showed a remarkably low blood sugar and during this same period the injected rabbits exhibited an increased sensitivity to the insulin. There was no apparent reason for this condition as there was no change in the treatment of the animals nor in their external appearance.

Autopsy showed no gross changes in the injected rabbits except a peculiar waxy appearance of the fat masses and a gray coloration of the adrenal cortex. No microscopical changes were observed. The work is being continued by Doctor Thatcher.

The inhibition of luminescence by light. A. R. MOORE, Physiological Laboratory of Rutgers University.

The coelenterates Mnemiopsis and Beroë, when dark adapted, show, upon stimulation, a blue green luminescence. Exposure of the animal to sunlight or to artificial light sufficiently prolonged causes the power of luminescence to be inhibited. Recovery occurs in the dark in about one-half hour. The quantity of light (intensity $\times$ time) which will just inhibit luminescence was found to be a constant, with a value for Mnemiopsis of 4,776 candle-meter-minutes, for Beroë of 57,285 candle-meter-minutes. The phenomenon therefore obeys the Bunsen-Roscoe photochemical law.

The dark adapted animal after mechanical stimulation for 20 seconds, no longer gives off light and must rest for half a minute before the power of luminescence is restored. We may therefore suppose that the luminescent substance A can be broken down by way of two reactions one of which is catalyzed by light, the other by excitation. These reactions are both reversible.

If we take the other view, namely, that light on the one hand and mechanical stimulation on the other, act by causing a destruction of the substance A, and that recovery must wait upon the formation of new molecules of A, then the following facts are not intelligible, but are to be understood only on the assumption of reversible reactions.

1. Peters found that mechanical stimulation accelerated the recovery of Mnemiopsis in which the power of luminescence had been suppressed by light. On the "destruction" theory the opposite result was to be expected.

2. Animals which have been fatigued by mechanical stimulation recover power of luminescence under exposure to light of strength sufficient to

inhibit luminescence in a few minutes. This proves the reversible character of the reaction by which the bioluminescence is produced. On the "destruction" theory, the light must destroy the potentially luminescent substance faster than it is formed, hence recovery from excitation-exhaustion would be impossible in the presence of light of such intensity.

It is known that light also affects the luminescence of the sulphides of zinc and calcium since previous illumination increases the brilliance of luminescence. On the other hand infra-red rays shorten the life of luminescence in zinc sulphide by causing a brief increase in luminescence and then a rapid decay. Neither of these effects of light can be observed in the luminescence of Ctenophores. There is therefore at present no analogy between the effects of light on the luminescence of the sulphides and on bioluminescence Ctenophores.

Augmentation of the vascular responses of the cat to successive vasomotor stimuli of equal values. BRENTON R. LUTZ and LELAND C. WYMAN, Boston University Medical School.

In a cat which has been etherized and pithed, stunned and pithed without an anesthetic, or anesthetized with urethane, successive vasomotor stimuli of equal values, such as equal doses of adrenalin or equal electrical stimulations of vasomotor nerves given over a period of several hours, evoke a series of vascular responses which are not equal in magnitude. A progressive augmentation of the vascular responses usually occurs, beginning about two hours after pithing, reaching a maximum in about three hours and disappearing in from three to four hours. This augmentation which varies between 30 and 600 per cent of the initial responses (averaging 100 per cent) has been attributed by various investigators to a sensitization of some part of the involuntary nervous system by a secretion from the thyroid gland or some other agency.

This augmentation of the vascular responses can occur after the adrenals and thyroids have been extirpated. It is not, therefore, due to hormone sensitization. It can occur in non-pithed animals, as well as in animals which have been pithed to various levels of the cord, even to complete destruction of the central nervous system. It does not depend on any particular level of blood pressure because it may appear in cases in which there is a fairly constant level, as low as 30 millimeters of mercury on the one hand and as high as 130 millimeters on the other. It is not due to variations in ventilation because this is kept constant by artificial respiration. Ether anesthetization before pithing delays the appearance of the augmentation, but is not its cause.

There is, however, a close correlation between the CO_2 capacity of the blood and these vascular responses. The onset of the augmentation is correlated with an increase of the CO_2 capacity which continues after the augmentation has begun to disappear. The CO_2 capacity later decreases to a low level. Thus there appears to be an optimum CO_2 capacity for vasomotor activity. The appearance of the augmentation can be prevented by keeping the CO_2 capacity low by the injection of HCl, and it can be brought on at will, either earlier or later than it usually occurs, by increasing the CO_2 capacity by the injection of sodium bicarbonate. In cases where an augmentation does not occur the blood pressure is low and falling and the CO_2 capacity either falls or remains at a level below that at which augmentation appears in other experiments.

Under the conditions which exist in a study of vascular responses by the methods described above there appears to be a temporary improvement of conditions in the tissues as suggested by the changes in CO_2 capacity. This improvement later gives way to a moribund condition which probably results from the abnormal vascular and respiratory conditions as indicated by the accompanying fall in CO_2 capacity. This suggests that under these circumstances there is an optimum pH for vascular responses, as would be expected from the work reported by various authors on the relation of pH to smooth muscle activity. It is further possible that these changes are indicative of variations in the ability of the tissues to utilize oxygen under the abnormal circulatory conditions. We prefer to interpret the augmentation in these terms rather than in terms of a sensitization of the autonomic nervous system.

The rate of blood flow as determined by a new method. HERRMANN L. BLUMGART and OTTO C. YENS, Boston City Hospital.

The rate at which blood circulates is of high importance in sustaining the nutrition of the tissues and maintaining the physiological integrity of the organism. The several methods at present available for estimating the rate of blood flow are either inadequate or untrustworthy. During the past seven months an attempt has been made to elaborate a more satisfactory approach to this fundamental problem of circulatory dynamics. The interest attached to this subject and the establishment of the validity of the principle we employed in these experiments would seem to justify this preliminary report of our progress.

The method utilized is as follows. The marginal ear vein and foot of a rabbit are the two points in the vascular circuit arbitrarily chosen between which the rate of blood flow is determined. The substance used for injection is the active deposit of radium. The active deposit is collected on salt, then dissolved in distilled water to approximate physiological saline solution, and finally injected intravenously. The syringe and needle are weighed before and after injection to determine the amount utilized. The animal is placed behind heavy lead castings of such thickness as to prevent emergent beta particles and gamma rays from reaching the detecting apparatus as the active deposit courses through the body toward the foot. The electroscope was first used for detection but subsequent experience proved that the apparatus described by Kovarik could be adapted to our needs with considerable advantage.

A hole one square inch in diameter is bored through the entire lead shielding. In the farther end of the hole, and away from the rabbit, lodges the ionization chamber. The hind foot of the rabbit is placed in the hole in front of the ionization chamber or detector. The arrival of the radium active deposit is signalled automatically by the emergent beta particles and gamma rays shooting from the blood vessels through the tissues of the foot into the ionization chamber. The air becomes a conductor of electricity, and the current flows across the air gap from the walls of the ionization chamber charged to 1400–1800 volts to the insulated and axially situated needle electrode. The current from the needle electrode is magnified by a three electrode vacuum amplifier and then used to operate a very sensitive relay. The relay closes a local battery circuit and thereby activates a recording pen galvanometer. An automatic registration of the time of arrival of the active deposit in the artery of the foot is thereby effected.

After this record is secured, the foot is withdrawn to a position behind the lead shielding, and in every instance a return to previously existing conditions is noted. In no experiment is any adjustment of the apparatus made from the beginning to the end of the run.

Up to now, our purpose has been to evolve a trustworthy method rather than to gain additional knowledge regarding the circulation. At present we are heightening the sensitivity of the detector and increasing the amplification of the small currents derived from it, in the hope of decreasing the necessary dosage of radium active deposit and so making the method clinically feasible.

The basic "metabolism" of excised muscle as affected by changes in the concentration of certain ions. The question of a response to adrenalin. E. H. BRUNQUIST, Department of Physiology and Pharmacology, University of Colorado School of Medicine.

The basic "metabolism" or surviving excised skeletal muscle (frog) was studied, *a*, with respect to the effect of qualitative and quantitative changes in the salt and hydrogen ion concentration of the immersion fluid, and *b*, with respect to a possible response to adrenalin.

The total acid production of the resting tissue was found to be relatively unchanged by considerable alterations in concentration of the salts of Ringer's fluid, or by the omission of some of them, but was profoundly affected by slight changes in the C_H.

On the working assumption that responsiveness to adrenalin,—inherent in the tissue *in situ*—might be lost incidental to excision, attempts were made, but without success, to "restore" favorable conditions: *a*, by providing a superabundance of oxygen; *b*, by addition of glucose or glycogen to the immersion fluid; and *c*, by supplying glutathione (in yeast extract).

Method of recording tension of muscles in situ with some preliminary results concerning the tensile relations of antagonistic muscles in reflex action. G. P. MCCOUCH, University of Pennsylvania.

Method: A flat steel spring similar in design to a pair of forceps has a small hole drilled through each arm close to the pivot. Two heavy waxed threads are passed one through each hole and sewed through and tied round the tendon which is then cut between the 2 arms of the spring. The shorter arm of the spring carries a small fibre block on which is mounted a nickel silver pointer flat above but rounded at the lower end. The pointer slides upon a German silver wire stretched between 2 contacts upon a second fibre block mounted at the end of the long arm of the spring at an angle of about 90°. Light insulated copper wire connects the 2 ends of the German silver wire with the derived circuit of a rheocord connected to a storage battery upon a potentiometer principle. The contacts upon the 2 arms of the spring are connected to the string of a Salamonson galvanometer. When no tension is put upon the spring the pointer on the short arm touches the contact on the long arm. When tension is applied the arms spread, the pointer sliding along the German silver wire. Thus the instrument acts as a potentiometer. The greater the tension developed the greater the current through the string of the galvanometer.

Conclusions from preliminary results: The tension changes in flexor and extensor muscles moving the ankle joint of the decerebrate cat in flexion and crossed extension reflexes vary with rate of movement.

Where movement is rapid, abrupt in onset, and of considerable range, as in the flexion reflex, reciprocal inhibition fails to keep pace with stretch and tension of antagonistic muscles rises, then drops to a plateau intermediate between its resting level and its peak. The peak is attained before excursion is complete. The prime movers attain a maximum tension within about 20 sigma of onset of movement which falls at a rate proportional to rate of movement to a low level from which there is rapid return, as excursion is completed, to an intermediate plateau level.

Where movement is slow, gradual in onset, and of minor range, as in a weak crossed extension reflex, some records indicate that reciprocal inhibition may exceed stretch, giving a fall in tension, may keep pace with it, giving no measurable change of tension, or if the reflex is more vigorous and of greater excursion may prove inadequate to prevent a gradual rise of tension usually followed by a fall to nearly resting level. Tension of prime movers rises in a gradual ascent, usually rounding into a slight fall in the terminal phase of excursion to rise again to a maximum which may be completely sustained.

Tension depends upon inertia and momentum, degree of leverage, and reciprocal innervation. The adjustment of reciprocal innervation through proprioceptive response to change of tension and of length may be studied by this technique under conditions which maintain the normal interplay of these mechanical factors.

Fluorescence and inhibition of luminescence of Ctenophores in ultraviolet light. E. NEWTON HARVEY, Princeton University and Nela Research Laboratories.

Ctenophores will not luminesce on stimulation after exposure to daylight or strong artificial light, but regain their power to luminesce again in the dark. This statement applies to the smallest bits of luminous tissue, even cells freed from the animal by agitation, that will pass filter paper.

Luminescence of the whole animal and of individual cells is suppressed by near ultraviolet light alone, without the visible, which can be removed by appropriate filters. In ultraviolet light alone the loss of luminescence on stimulation can be watched during exposure, and it is observed that a resting animal is not stimulated to luminesce by the ultraviolet light. Therefore inhibition of luminescence by light is not akin to fatigue, due to oxidation of the store of photogenic material.

Ctenophores stimulated several times and then placed in ultraviolet light show a persistent luminescence in the same position as the luminescence due to stimulation. This persistent luminescence disappears instantly in the dark and reappears instantly in the ultraviolet light. It is not obtained with light adapted Ctenophores and is interpreted to be a fluorescence of the oxidation product of photogenic material.

Marked fluorescence of the luminous organ of the glow-worm and of the luminous slime of the annelid, Chaetopterus, is observed in ultraviolet light, but no marked fluorescence of the luminous substances of Cypridina. Nevertheless, chemiluminescence seems to be connected with fluorescence in living things as in the unsaturated silicon compounds and the Grignard reagents.

The asynchronism of the contraction process in the right and left ventricles. LOUIS N. KATZ (introduced by C. J. WIGGERS), Western Reserve University Medical School.

It is generally assumed, though without much experimental backing, that the two ventricles initiate and terminate their contractions simultaneously. This question was reinvestigated in this research by precise methods. The two intraventricular pressures or the two aortic pressures just outside the semilunar valves were recorded simultaneously by optical manometers, especial attention being paid to parallax. From the former curves the relative onsets of the isometric contractions and the ends of systole were calculated; from the latter the moments of ejection and the end of systolic ejection were determined.

The following results were obtained: 1, Under normal experimental conditions neither the onset of the isometric contractions, the beginning of ejections nor the end of systole were synchronous events in the two ventricles. In 24 selected experiments, reported at this time, the contraction of the right ventricle preceded the left in 11 instances and approximately by 0.016 to 0.027 second. Of these experiments, it was found that right ventricular contraction terminated earlier in 3 cases by 0.01 to 0.02 second but terminated later by about 0.015 second in 8 cases. Consequently the duration of the right ventricular contraction was longer than the left in 8 experiments while it was of shorter duration in 3 experiments. In 11 other experiments ventricular systole began later on the right side by 0.013 to 0.03 second. In these experiments right ventricular systole also ended later by 0.016 to 0.026 second in 10 cases while in the remaining one they ended simultaneously. Right ventricular systole was longer than the left in 4 of these cases, of equal length in 4 cases and shorter in 3 cases.

2. When the heart was slowed by vagus stimulation the degree of asynchronism at the beginning and end altered from beat to beat thus varying the disproportion in right and left sided contraction periods. In the majority of the experiments right ventricular systole and ejection began relatively earlier during stimulation than in the controls. In the larger number of cases the right ventricular systole and ejection tended to end relatively later during stimulation than in the controls and this occurred very often in spite of the opposite tendency of the onset of contraction (or ejection). The net result was that in most cases the lengthening of systole and ejection was greater on the right side; although in a small number of cases the reverse effects were noted.

3. When the initial volume and tension in the ventricle were increased by saline infusion the durations of systole lengthened. Usually, but by no means always the right ventricular systole lengthened more than the left. In some experiments this was preceded by a primary shortening of the contraction phases which affected the left ventricle more than the right. In some instances these differences in the duration of right and left sided contractions were partly due to slight changes in the relative beginning of contraction but was always predominantly and sometimes entirely due to differences in the termination of right and left sided contraction.

These variations in the asynchronism beginnings and endings of right and left sided ventricular contractions and others produced during partial asphyxia, during partial compression of the aorta, during partial compression of the pulmonary vessels, during different degrees of lung inflations, all lead to the following conclusions:

1. In experimental dogs right and left ventricular systoles neither begin

nor cease to contract simultaneously, nor is the length of the two systoles equal.

2. The asynchronism of the beginning and end of contraction varies slightly during consecutive beats but is changed markedly or often entirely reversed during vagus stimulation and after, by venous infusion and other experimental procedures.

3. The effect of this is that the duration of right and left ventricular systole are neither equal nor do they change proportionally or constantly when influences operate which change the duration of these contractions.

The maximum of human power, and the fuel of muscular work: From observations on the Olympic Championship Crew of 1924. YANDELL HENDERSON and HOWARD W. HAGGARD, Yale University.

Rowing in a racing shell with sliding seats probably allows a nearer approach to maximal work, by more nearly all the muscles of the body, than any other form of athletics. The eight men who rowed in the Yale University boat in 1924 demonstrated in a series of races, ranging from a mile and a quarter up to four miles, that for all these distances they were able to lead any other crew in their own country. After winning the right to represent America in the Olympic games at Paris, they won in the trial heats and led by several lengths in the final race on the Seine, winning from crews from all parts of the world and establishing a world's record for the 2000 meter course.

During the season of training from January to June, 1924, we were fortunately able to make from time to time on five of these men determinations of the respiratory exchange and quotient, the oxygen consumption and deficit, the CO_2 output, etc. The external work was also determined by means of a rowing machine set up in the laboratory and arranged as an ergometer. Later a determination of the power necessary to drive a racing boat at various speeds was obtained by towing it by means of a power boat with the tow line fastened to a spring balance. The figures from the draw bar pull multiplied by the speed in feet per minute give the absolute net work which the crew has to do. An additional twenty-five per cent of external work was assumed, in order to cover the energy expended in moving the slide and oar in returning to the stroke position, and was added to the results from the rowing machine and draw bar pull methods.

The data from these three methods were in general in fair agreement. They indicate that the maximal power exerted is from 0.45 to 0.55 horse power per man, or expressed in the heat equivalents, 4.8 to 5.9 calories per minute, with a total energy expenditure of 19 to 29 calories per minute, or 13 to 20 times the basal rate. The power expressed by the smaller of each of these pairs of figures is that maintained, and is therefore approximately the maximum that a man can maintain, for 22 minutes during a four mile race; while the higher figures are applicable to the more intense exertion and greater speed, which are also maximal, for about six minutes in races of about one and one-third miles or 2000 meters. The corresponding figures for the volume of oxygen consumed per minute are 3.5 and 4 liters; the latter figure is about the limit of the transporting capacity of the lungs, blood, and heart. An oarsman exerts a power, which exceeds by 30 to 60 per cent that afforded by the oxygen simultaneously absorbed; he thus draws heavily on his credit, and incurs oxygen deficits of 4 to 8 liters or more, and these deficits are repaid by the high rate of oxygen absorption

for a time after the work is ended. This is in accord with A. V. Hill's conception.

The most significant result of these observations is the evidence which they afford, in general agreement with Krogh and Lindhard, but in disaccord with the Hill-Meyerhof conception in its original form, that in whatever proportion fat and sugar are being burned during rest just before the exercise, they are burned in nearly the same proportion to produce the energy for doing work and for recovery. Thus it is found that in these oarsmen the respiratory quotients for the work and recovery periods combined were approximately the same as those during rest before the work. In one experiment the man had missed his breakfast and eaten nothing for eighteen hours; yet he made an intense exertion, although rather disadvantageously, on a combustion almost entirely of fat from his own body. His respiratory quotient during rest was 0.75, during 3 minutes of rowing on the machine it was 0.72, and during 10 minutes' rest afterward, 0.73. The work done was equivalent to 6 calories per minute, the total energy expenditure 29 calories per minute; this man's weight was 180 pounds (82 kilos) and his height 6 feet 4 inches (193 cm.). In general the observations show that much more than half the energy expended by these athletes in muscular work is drawn from fat, and much less than half from sugar. A much larger proportion of carbohydrates would probably be advantageous.

In contrast to the effects of great exertion on untrained men, there was in the members of this crew only a slight overbreathing, or sometimes practically none at all, with a correspondingly slight blowing off of CO_2 during work or afterward. Apparently some of the phenomena, especially the blowing off of CO_2 and the high respiratory quotient during and immediately after intense exertion, which are commonly explained as due to the development of an "acidosis," and which Hill has instanced as in accord with the Hill-Meyerhof conception of muscular contraction, are due to the stimulation of the respiratory nervous regulation by oxygen deficiency in the arterial blood rather than to displacement of carbonic acid from the blood carbonates by lactic acid.

Higher nervous activity in the thyroidectomized sheep and the effect of thyroxin thereon. HOWARD S. LIDDELL (by invitation) Department of Physiology, Cornell University Medical College, Ithaca N. Y. (Fellow of National Research Council).

Twin sheep, one of which was thyroidectomized, were caused to learn to escape from a simple maze of three parallel alleys. Either outer alley could be made a cul de sac. When the animals were taught to escape, the position of the blind alley being reversed at every trial, distinct differences were observed between the behavior of the thyroidectomized sheep and its control. Frequent pauses were made by the cretin in running the maze even after it was familiar with the correct path. These pauses are not due to fatigue because shortening the alleys does not abolish them. They have also been observed to appear in a thyroidectomized sheep when it ceased turning mechanically, always in the same direction, and began to alternate. When a wrong turn is inhibited for the first time a pause often replaces the incorrect response even in the normal sheep. More trials are required by the thyroidectomized animal to acquire the alternation habit than by the control and the habit is much less stable. Thyroxin,

although it increases the spontaneous activity of the cretin, does not abolish its pauses in the alternating maze. It seems, however, to increase the stability of such a habit.

The effects of internal and external pH on the penetration of arsenic from arsenate and arsenite solutions into a living cell. MATILDA MOLDENHAUER BROOKS, Hygienic Laboratory, Washington, D. C.

Analyses of the wall, protoplasm and sap of cells of the large unicellular marine alga *Valonia macrophysa* showed that the amount of arsenic found in these tissues after immersion of the cells in arsenate or arsenite solutions depended upon the pH of the solution and upon that of the interior of the cell. The pH of the sap, and presumably that of the protoplasm also was varied by previous treatment of the cells with $NaHCO_3$ or NH_4Cl.

The concentration of arsenic in the sap was invariably less than that in the protoplasm, both of which contained the minimum amount of arsenic when the external pH was about 7.0 to 7.5, other conditions being constant. Either acidity or alkalinity of the outside solution (pH 5.0 or 9.0) markedly increased the amount of pentavalent arsenic found, whereas in the case of trivalent arsenic only alkalinity had this marked effect. These differences cannot be explained by differences in dissociation of arsenic and arsenious acids at the various H ion concentrations.

The effects of internal pH also varied with the valency of the arsenic; the penetration of the arsenate anion was increased by increasing the hydrion concentration and vice versa, while that of the arsenite anion was affected in the opposite way.

Variations in the kidney related to dietary factors.[1] THOMAS B. OSBORNE, LAFAYETTE B. MENDEL, EDWARDS A. PARK and MILTON C. WINTERNITZ, Yale University Medical School.

There is a widespread belief that a high-protein diet in man is a renal irritant and that it may be associated with the development of nephritis. Inasmuch as rats will grow on rations extremely rich in protein, provided all other dietary essentials are supplied, it has become possible to study the development of the organs under such conditions. On high-protein diets the kidneys become strikingly enlarged, the increments in size often exceeding 50 per cent of the normal weight of these organs. The preliminary histological examinations have failed to disclose changes of an inflammatory or degenerative nature. The renal enlargement has occurred without hypertrophy of the heart. Comparable changes in kidney size can be brought about by the inclusion of large quantities of urea in the diets. The excretory functions of the kidneys have been greatly augmented by the administration of large quantities of a variety of inorganic salts without, however, bringing about the hypertrophy that develops through the necessity of eliminating the nitrogenous waste. The rapidity with which the kidneys respond, by enlargement, to large increments of protein in the diet is surprising; in many instances marked changes have resulted within a week. When the ration is changed to a lower protein level after a regimen of high protein that experience has shown to be adequate to produce the hypertrophic changes, a retrogression in the size of the kidney ensues.

[1] The expenses of this investigation were defrayed in part by the Carnegie Institution of Washington.

The relation of the cerebral cortex to extensor rigidity. J. M. D. Olmsted, University of Toronto.

Portions of the cerebral cortex around the sulcus cruciatus of cats were removed aseptically from one side of the brain. When the injury involved both margins of the sulcus the cat showed flaccid paralysis on the opposite side of the body. Complete recovery from paralysis took place within a week. But it was found that if the cat was held up off the ground, the limbs, especially the hind one, on the side opposite the injury remained in a condition of extensor rigidity. This condition was permanent, at least for 3 to 4 months. If the lower margin of the sulcus was injured toward the midline, no paralysis followed, but the extension phenomenon was present. If the outer and upper margin was injured only flaccid paralysis resulted.

Rhythmic contractions in strips of mammalian ventricle. Helen B. Taussig and Faith Meserve, Boston University.

These contractions were obtained by immersing isolated strips of mammalian ventricular muscle in oxygenated isotonic solutions. That ventricular strips from cold blooded hearts may be made to beat rhythmically after immersion in such solutions is well known. Hitherto, it has been believed necessary for the isolated mammalian ventricle, or strips therefrom, to be perfused through the coronary vessels in order to obtain rhythmic contractions.

We have obtained by simple immersion spontaneous rhythmic contractions from virtually all portions of the ventricle. Contractions of this kind were obtained in experiments on the cat from the papillary muscle, septal wall, free wall, deep circular layer, apex, and external layer of the left ventricle and the moderator band of the right ventricle. Results of the same nature were obtained from heart strips of the dog, rabbit, calf, sheep, and human autopsy material.

The technique is essentially the same as that used for cold-blooded heart strips. The mammalian strips were immersed in the fluid at a low temperature under very slight tension.

Ringer's, Tyrode's, or Locke's solution were used as an immersion fluid. From 5 to 10 per cent of defibrinated blood aided in eliciting contractions, though a high proportion of blood was apparently not advantageous. The optimum temperature was between 32 and 35 degrees C. Oxygen is essential for the prolonged continuation of contractions.

For the stimulation of a non-beating muscle strip the usual mechanical and electrical methods have been successful. These frequently elicit a brief series of rhythmic contractions, but rarely a series of long duration. On the other hand, a muscle strip from a cat heart which responds but temporarily to electrical stimulation will respond to a solution of adrenalin (1 drop of 1:1000 dilution of Parke Davis Co. preparation) with a lasting series of rhythmic contractions.

Practically all of the variations observed by other workers on turtle ventricular strips have been observed by us in mammalian ventricular strips. We have seen pulsu salternans, grouped beats, Lucianic periods, various irregularities in the extent of contraction, and true tonus waves similar to those not infrequently observed in strips from turtle auricle.

Finally, rhythmic contractions have been observed in strips from the specialized tissue of the calf heart, which on sectioning proved to be

mainly Purkinje tissue but contained a very small amount of cardiac muscle. Minute strands from the interior of the dog's heart composed principally of the specialized tissue have likewise given spontaneous rhythmic contractions. This phase of the investigation is being continued.

The influence of insulin on the respiratory metabolism of normal rabbits. ESTELLE E. HAWLEY (introduced by John R. Murlin), Department of Vital Economics, University of Rochester, Rochester, N. Y.

The observations to date on the respiratory changes resulting in normal individuals from insulin injections are conflicting. It is generally agreed that insulin causes a rise in respiratory quotient when administered to normal subjects but just how this change is brought about is still a matter of discussion. Is the increase in quotient due to increase in CO_2, decrease in O_2, or both?

Normal healthy rabbits were used as the experimental animals in this series of experiments. All experiments were carried out under the same conditions as to care, diet, and time elapsing between food and experiments. Great effort was made to obtain comparable results. The blood sugar curve coincident with the respiratory change was followed. This also served as a check on the potency of the insulin.

Control alcohol checks were made at regular intervals and any variation of more than 0.01 from the theoretical 0.667 quotient was investigated and the necessary correction made before further data were obtained. The only results which were considered satisfactory were those which could be verified by a theoretical quotient from alcohol. From the findings to date the following summary seems justified:

1. Insulin when administered to normal animals brings about, first a slight decrease, then a decided increase in the R. Q. curve, reaching the peak two hours after injection and returning to the basal level four hours after.

2. A blood sugar drop accompanies the R. Q. change, the lowest blood sugar occurring at the peak of the R. Q. curve. The return to normal is less rapid than the return to normal of the R. Q. though the curve is well on its return by the end of four hours.

3. There is, in the second hour, both a decrease in the O_2 and an increase in the CO_2, both changes tending to increase the R. Q.

4. The O_2 consumption and the heat production would indicate that the total metabolism is not markedly increased.

Energy metabolism of full-term and premature infants with special reference to the influence of food and crying. M. ELIZABETH MARSH (introduced by JOHN R. MURLIN), Department of Vital Economics, University of Rochester, Rochester, N. Y.

Two hundred and thirty-four observation periods were made upon fifty normal newborn infants ranging in age from six hours to fifteen days but of this number only ninety-eight periods with thirty-eight infants were truly basal, i.e., when the infant slept quietly throughout.

The basal metabolism of these thirty-eight infants averaged 6.67 calories per hour or 2.00 calories per kilogram and 29.16 calories per square meter per hour (Lissauer formula) but varied with age, being the highest in the second twenty-four hours and on the basis of surface falling gradually to the sixth day from which point it rose steadily.

The respiratory quotient in these basal periods ranges from 0.66 to 1.16.

The average of all quotients whether basal or not is from 0.79 the first twenty-four hours to 0.75 on the fourth and thence gradually upward to 0.85 on the ninth day.

Applying statistical methods it is found that surface area is, as usual, a slightly better measure of basal metabolism than body weight and furthermore there is practically no correlation between heat production and pulse rate.

With regard to the influence of crying on energy metabolism, the interesting observation is made that in the average infant, active, healthy crying requires just as much again expenditure of energy as the basal metabolism. Expressed differently, crying one per cent of the time raises the metabolism one per cent.

Especial attention was given to the effects of natural food and of supplementary feedings of lactose and dextrose. It was found particularly difficult to raise the respiratory quotient by means of supplementary feedings on the second and third days and likewise the dynamic action of ordinary and of supplementary feedings within the first eight days is very small. The largest recorded was twelve per cent from a feeding of ten per cent lactose. Dextrose was about the same as lactose. Comparing the effects of small feedings (averaging 26.7 grams) of food or the sugar solution with that of large feedings (averaging 51 grams) the increase in the basal heat production averaged approximately seven per cent.

In eighty-two observation periods upon twenty-one infants whose prematurity ranged from two weeks to two months, the most striking difference was the lowered heat production, the basal metabolism being 2.91 calories lower when measured per square meter per hour. The influence of food and of activity was practically the same as in the full-term infants.

This study will be published in full in the American Journal of Diseases of Children.

Proof that the hot summer weather increases and the cold winter weather decreases the catalase content of the needles of evergreen trees corresponding with an increase and decrease produced in their respiratory metabolism.

W. E. Burge, Physiological Laboratory, University of Illinois.

Needles of several species of pines growing in this section of the state of Illinois were used in this investigation. The catalase content of these needles was made weekly throughout the whole of the present year. After collecting and grinding the needles through a small hashing machine twice, one gram of this material was added to 25 cc. of neutral hydrogen peroxide and the amount of oxygen liberated in ten minutes was taken as a measure of the catalase content of the needles. Only results obtained from the needles of the white pine (P. Strobus) will be reported in this paper since these were typical.

The average amount of oxygen liberated by the needles of this pine during the month of January was 34 cc.; during February, 36 cc.; during March, 40 cc.; during April, 82 cc.; during May, 120 cc.; during June, 136 cc.; during September, 68 cc.; during October, 65 cc.; during November, 46 cc. The average temperature for January was 21°F.; February 30°F.; March 36°F.; April 54°F.; May, 57°F.; June, 70°F.; September, 61°F.; October, 60°F.; November, 47°F.

From the preceding data, it may be seen that the catalase content of the needles was the lowest during the months of January and February when

the weather was the coldest and as it became warmer, passing from winter into spring and summer, the catalase rose and was highest in the summer when the temperature was the highest. As the weather became cooler, passing from summer into fall and winter, the catalase content decreased and by November had returned almost to the level found during the previous January. These observations are in keeping with the fact that the respiratory metabolism of the pine needles is lowest in the winter when the weather is coldest, and highest in the summer when the weather is hottest.

The persistence of experimental suprarenal adynamia. E. P. DURRANT (introduced by R. G. HOSKINS), Ohio State University.

In a previous study of the effect of adrenal deficiency on bodily vigor it was found that a marked degree of adynamia was maintained throughout the experimental period. In the series studied by the revolving cage method the maximum period of observation was seven weeks. A second series is reported in which the revolving cage method was used. Twenty-two epinephrectomized rats and seventeen controls were studied over a period of seven to sixteen weeks. All showed some degree of adynamia. Five maintained marked adynamia throughout the period. Ten partially recovered. Seven, within seven to thirty-five days, became as active as their controls. The relation of residual adrenal tissue to recovery is considered.

The effect of thyroidectomy and thyroid feeding on the oestrous cycle in the white rat. M. O. LEE (introduced by R. G. HOSKINS), Ohio State University.

The effect of thyroidectomy on the oestrous cycle in the adult albino rat was studied by the vaginal smear method. In 10 of 12 cases either the whole cycle was shortened in time or the period of oestrus (stage II) was considerably prolonged. In the latter condition some of the animals remained in the "heat" stage for as long as twelve days, as evidenced by 1, the microscopic picture presented by the smears, and 2, the reactions of the animal when placed with a male. This condition was relieved by thyroid gland feeding, during which the cycles approached normal. Thyroid feeding of normal rats caused a slight but consistent lengthening of the total cycle, but not of the oestrous stage.

The effect of inhalation of ether on the intestine of rabbits and dogs. A. E. GUENTHER and MELVIN W. BINGER (by invitation), College of Medicine, University of Nebraska, Omaha.

The inhalation of ether by an animal under light anesthesia results in an increase in tonus of the small intestine, evidenced by a slow rise of the base line in a record of intestinal activity. Superimposed upon the base line are the usual oscillations obtained when a writing lever is attached to an active intestine. These, too, are somewhat augmented in height but their rate is unchanged. The stimulating effect of ether is not present in deep narcosis; rather, the reverse. The records were obtained by use of a modified Trendelenburg method.

The increased tonus by ether is brought about by an action peripherally on nervous structures in the intestinal wall. Destruction of the thoracic cord, section of the vagi, or both, does not alter the response. Clamping

the abdominal aorta or otherwise excluding the blood stream from the intestine totally abolishes the effect even though the innervation be intact. An atmosphere of ether vapor surrounding the intestinal segment increases its tone but with greater concentration finally depresses all activity. Occlusion of the trachea does not produce the ether effect. The administration of atropin in proper dosage converts the ether effect from a rise in tonus to a corresponding fall. The reaction of the intestine to electrical stimulation under the above conditions parallels the effect produced by ether inhalation. If faradic stimulation produces a rise of the lever, then ether will; if electrical stimulation produces a fall, then ether will likewise produce a fall. This alteration in response, after atropin administration, is assumed to be due to the paralysis of the bulbar autonomics by the atropin, leaving, for a time, at least, the sympathetic stimulation by the ether unopposed.

Effects of spleen and marrow extracts on blood coagulation in normal humans.

C. D. LEAKE, University of Wisconsin.

It has been shown in a series of experiments reported elsewhere, that the administration of spleen and red bone marrow in rabbits, dogs, and humans, stimulates red blood cell production to a marked degree. Moreover, it was definitely indicated that they are more effective as hemopoietic agents when used in combination than when employed singly. While the rise in hemoglobin content is not as rapid nor as great as in the case of the number of circulating erythrocytes, it is better sustained when administration is stopped. Determinations of blood volume show that the findings are not due to blood concentration. The number of reticulocytes does not increase sufficiently under the influence of the combined materials to account completely for the total rise in erythrocytes. The discrepancy is partially explained by the observation that the resistance of the red cells to hypotonic saline solutions is augmented after the ingestion of the spleen and marrow compound.

Using various clinical methods, it appears that the daily oral administration of 1 gram of desiccated spleen and red bone marrow combined in equal proportions by weight, reduces coagulation time in normal humans by 20 to 30 per cent. A more careful study, using the technique proposed by Gibbs,[1] confirmed this finding in all but one of fifteen normal subjects. In this atypical subject, the coagulation time, which was short to begin with, remained constant. In the others, the coagulation time returned to normal a few days after administration was stopped, to fall again when administration was resumed. These effects were usually apparent about two days after the administration was begun, or about a day after the initial rise in the red cell count. The results of experiments not yet complete suggest that the desiccated marrow is more responsible for the effect than the spleen in the combination used. Since the desiccated marrow contains considerable calcium, control experiments were made in which equivalent amounts of calcium lactate were given by mouth. No significant changes in coagulation time resulted.

Determinations of the blood platelets in normal humans indicate that the number of these bodies is increased after the ingestion of the spleen and marrow mixture. In this case again, the administration of marrow

[1] Quart. Journ. Med., 1924, xvii, 312.

alone seems to be more effective than spleen alone, but not as effective as the two combined.

The effect of changes in position of the heart on the Q R S complex of the electrocardiogram. WALTER J. MEEK and J. ALLEN WILSON (by invitation), University of Wisconsin.

Certain modifications of the electrocardiogram which appear coincidently with changes in posture or with the phases of respiration have long been attributed to variations in the cardiac position. Lateral displacements of the heart due to pleural effusions, air or adhesions may also modify the curves. The bearing of these phenomena on interpretation of the normal and clinical electrocardiograms is obvious. In man the heart thorax relationships cannot of course be altered experimentally although several workers have varied the position of the leads and then cleverly imitated changes in heart position. Animal experimentation has not been used to any extent in this problem due to the general feeling that manipulations with the chest open made it impossible to secure records sufficiently normal to be of value. This fear we have found not to be justified. By means of ligatures around the pericardium at the apex of the heart and small hooks in the epicardium various positions of the heart could be secured. The records were usually close approximations of normal curves and often identical with them. The electrocardiograms were made by taking either two or three leads simultaneously. The following results have been noted.

1. Displacements of the heart around its antero-posterior axis either to the right or left as carried out experimentally do not give the curves anticipated according to the principle of the Einthoven triangle or according to the accepted standards for right and left sided preponderance. The electrical axis may be either increased or decreased or remain the same.

2. If when the heart is displaced to the right or left a counter rotation in the opposite direction on its long axis is brought about the curves approach those of right and left sided preponderance, the electrical angle regularly increasing or decreasing as it should.

3. It is evident that the effects of mere displacement have been masked by rotation on the longitudinal axis. In proof of this it is found that if the heart is held in its normal position and then rotated on its long axis, typical curves of right or left sided preponderance may be produced.

4. Either uncomplicated rotation to the right on the anteroposterior axis, or rotation to the left on the longitudinal axis will give curves characteristic of right sided preponderance. The reverse conditions will give curves characteristic of left sided preponderance.

5. The importance of having the heart in the normal position for all EKGs is emphasized. It seems clear that it is impossible to tell how much a change in position may affect the EKG or how much an abnormal EKG may be due to variations in position, since the result is due to the summation of different rotations.

Stimulating efficiency of intermittent light and its bearing on the nature of stimulation. S. O. MAST, The Johns Hopkins University, and WM. L. DOLLEY, Randolph Macon College.

At a high flash-frequency the stimulating efficiency of intermittent light

is equal to that of continuous light. If, beginning at this rate, the flash-frequency is gradually decreased, the stimulating efficiency increases to a maximum which is much higher after which it decreases to a minimum which is much lower than that of continuous light. The higher the luminous intensity, the higher the flash-frequency required to make the stimulating efficiency of intermittent equal to that of continuous light.

The flash-frequency for maximum stimulating efficiency also varies with the luminous intensity. It is approximately 33 per second in 550 m.c.; 25 in 227 m.c.; 20 in 92.19 m.c.; and 14 in 9.46 m.c.

The stimulating efficiency depends upon the ratio between the length of the light and the dark periods. At optimum flash-frequency it is somewhat higher, if the dark periods are three times as long as the light periods, than it is if they are 10 or 15 times as long and considerably higher than it is if they are the same in length. If the dark periods are four times as long as the light periods the stimulating efficiency of intermittent light is, at all flash-frequencies tested, about equal to that of continuous light.

The stimulating efficiency of light with a flash-frequency of 20 per second, an intensity of 115 m.c. and the dark periods three times as long as the light periods, is between 16 and 22 times as great as that of continuous light. That is, under optimum conditions, it requires to produce a given effect with continuous light more than 16 times as much energy as it does to produce the same effect with intermittent light.

These results indicate that stimulation in continuous light is not a continuous process and that there are in the nervous system alternate sensitive and refractory periods. They indicate that beginning immediately after the organism is illuminated light acts for a short time inducing certain changes, after which it ceases to act and reverse changes take place, which may be thought of as a process of restitution, and that after these processes are complete and the system has assumed its former condition or nearly so, light acts again for a time, after which restitution again occurs, etc., the intervals during which light acts being the sensitive periods, and those during which restitution occurs the refractory periods.

Data concerning plurisegmental innervation. GEORGE D. SHAFER and PERCY G. STILES, Harvard University.

Evidence has been secured by Cattell and Stiles that nearly all the fibers of the gastrocnemius of the frog can be thrown into contraction by stimulating either of the two main divisions of the sciatic nerve. When the muscle is responding to one of these little is gained by adding the influence of the other. In the experiments previously reported the induction shocks applied to the two components of the nerve had invariably been derived from a single coil and had, accordingly, been synchronous. It seemed desirable to ascertain whether any gain would be secured by employing two coils and making the shocks successive. It was thought possible that with two approaches to a muscle-fiber available the second might be advantageously utilized while the first had fallen into a refractory phase. Spacing the stimuli by means of a rotary interruptor we have failed to find any such summation. Negative results have persisted for all intervals throughout a wide range.

[1] This Journal, 1924, lxix, 645.

The coagulation of egg albumin by ultraviolet light and heat. JANET HOWELL CLARK, School of Hygiene, Johns Hopkins University.

Dialysed egg albumin solutions, brought to different hydrogen ion concentrations by means of dilute hydrochloric acid or sodium hydroxide, were radiated with ultraviolet light or heated to 100°C., and the following results were obtained. At any pH less than 4.8 (the isoelectric point) the radiated solutions remained clear but showed a reversible coagulation on being brought to pH 4.8 to 6.8, and exactly similar results were obtained on heating. On radiating or heating solutions between pH 4.8 and 5.2 a flaky precipitate was formed and previous radiation did not inhibit heat coagulation. Solutions radiated or heated between pH 5.2 and 6.2 became opalescent and radiation prevented further coagulation on subsequent heating. Solutions heated between pH 6.2 and 8.0 showed a faint opalescence. Solutions radiated at a pH greater than 6.2 remained clear but the charge on the colloidal particles was changed from negative to positive and the radiated solutions were desensitized to heat coagulation. When radiated at 0° the solutions showed no visible coagulation except a faint trace of opalescence between pH 4.8 and 5.2 but they showed the same change in charge and the same desensitization to heat coagulation as when radiated at room temperature.

From these and other results it was concluded that coagulation by ultraviolet light is initiated by a discharge of electrons on the absorption of light energy, which takes place at any hydrogen ion concentration and at any temperature. At temperatures greater than 0°, and for a pH between 4.8 and 6.2, this photo-electric effect is followed by a dehydration of the protein molecule, with the formation of a visible coagulum. Heat coagulation is supposed to be due to a dehydration which is inhibited by previous radiation when light and heat attack different parts of the molecule.

Electrical stimulation of luminescence—A case of reversed Pflüger's law. A. R. MOORE, Physiological Laboratory of Rutgers University.

When subjected to the action of the galvanic current the luminescent Ctenophores, Mnemiopsis and Beroë, respond on the make of the current with a luminescent glow on the anodal side which persists for several seconds during the flow of the current. On the break, Mnemiopsis reacts with a flash on the cathodal side. This constitutes a case of "reversed Pflüger's law." If a transverse incision be made in the animal, a secondary anode is observed at the cut surface when the current is passed. This proves that the locus of stimulation is at the protoplasm-sea-water surface. The spark discharge of an influence machine is practically without effect. The rôle of ions in galvanic stimulation of the Ctenophores was shown by putting a specimen into a trough containing a solution of pure NaCl, and testing with the constant current. No stimulation of luminescence occurred although the animal responded to mechanical stimulation in the usual way. But when $CaCl_2$ was added to the solution in the ratio 1 mol $CaCl_2$ to 500 NaCl then the electric current caused luminescence at the anode on the make. Tonic stimulation of luminescence by means of solutions of pure salts (isosmotic with sea water, pH 7.8) was obtained with $CaCl_2$, $SrCl_2$, $BaCl_2$ and KCl, but not with NaCl and $MgCl_2$. Therefore, not only is Pflüger's law reversed in the case of electrical stimulation of luminescence but also the ionic basis of that law is reversed, since stimulation depends upon a decrease and not an increase of the ratio $\frac{C_{Na}}{C_{Ca}}$.

The influence of feeding pituitary gland (hypophysis) on the growth and development of the flesh flies. T. L. PATTERSON, Detroit College of Medicine and Surgery.

The alleged influence on the growth and rate of development from feeding pituitary gland substance has been principally studied on vertebrates, and for this reason, it seemed desirable to determine how some of the intertebrates would react to this gland substance, in whose body no organ comparable to an endocrine gland has, so far as I know, been definitely demonstrated.

Flesh flies were used in the investigation and the majority of the experiments were carried out on a larviparous fly of a *Sarcophaga* species, probably *saracena*. These flies may be caught easily on the window ledges of any laboratory during the summer months. They are grayish in color and can usually be distinguished by the checkerboard pattern of gray and black squares on the abdomen. They were paired and placed in separate wire cages for rearing young. First stage larvae were obtained shortly after death of the female fly by cesarean section for these larvae or maggots will feed on the tissues of the mother fly in case putrid material is unavailable for larviposition. The larvae so obtained were equally distributed on slightly decomposed pituitary substance consisting of anterior lobe, posterior lobe and whole gland and control substances consisting of brain and muscle, all being obtained from the hog. These five respective substances were contained in glass tubes of about 2.5 grams' capacity to prevent drying. In approximately 5 days the larval history was completed and the animals had passed into the pupal stage in which condition they could be weighed and measured. A species of *Calliphora*, probably *erythrocephala*, an oviparous fly was used in a few experiments.

The results of these experiments indicate that there is no growth producing substance present in the pituitary, not even in the anterior lobe when fed under the conditions described to flesh flies since it causes no increase in growth or in the rate of metamorphosis over that of the controls. The slight variations that do occur may be assumed to fall within the limit of experimental error or to variations in the rate of decomposition of the respective tissue substance fed.

Viscero-motor reflexes. FREDERICK R. MILLER and H. M. SIMPSON, University of Western Ontario.

The decapitate cat of Sherrington was used in this study of viscero-motor reflexes, a complete account of which appeared in Trans. Royal Soc. Canada, sec. v, 1924, xviii, 147.

It was shown that in these reflexes not only are the abdominal muscles involved as effectors, as shown by Sherrington and Mackenzie, but the muscles of the hindlimbs also participate. The hindlimb reactions are of a defensive character. Attention was previously called to this characteristic in the frog and turtle by Carlson and Luckhardt.

In the course of our work it was found that distension of the stomach with air elicited reflexly alternating movements in the hindlegs and contraction of the abdominal muscles. Traction on the stomach and its mesentery and the tying of ligatures in the stomach wall yielded similar reflexes.

Chemical stimulation of the gastric mucosa with mustard oil caused

marked "facilitation" of the hindleg reflexes (flexion, crossed extension and kicking). Lashing of the tail often occurred. Sometimes great activity of the hindlegs was manifested apart from stimulation applied directly to them. Powerful abdominal rigidity also occurred. That the afferent impulses were being conveyed by the splanchnic nerves was proved by the abolition of the gastric reflex influences as a result of excision of the coeliac ganglia and the superior mesenteric ganglion. Faradisation or mechanical stimulaton of the central ends of the gastric visceral nerves evoked abdominal rigidity, hindleg reflexes and a type of cremasteric reflex.

Stretching or squeezing a loop of small intestine, also traction on its mesentery, yielded hindleg reflexes and abdominal rigidity. Similar reflexes were evoked by faradisation or mechanical stimulation of the central ends of the superior mesenteric nerves.

Faradisation of the central ends of the hepatic nerves yielded hindlimb reflexes and abdominal rigidity, most intense on the right side.

Mechanical stimulation of the spleen evoked hindlimb reflexes and left-sided abdominal rigidity. Similar effects were noted on faradisation of the central ends of the splenic nerves.

Mechanical stimulation of the kidney yielded hindleg reflexes with ipsilateral abdominal rigidity. Faradisation of the central end of the hypogastric nerve yielded hindleg reflexes with abdominal rigidity.

The motor responses in these reflexes constitute the outward signs of pain sensations and afford a means of studying visceral sensibility objectively. After division of the dorsal roots of the 7th, 8th, 9th and 10th thoracic spinal nerves it was found that the hindleg and abdominal reflexes previously elicitable from the gastric visceral nerves were almost completely abolished. These results are in general agreement with the views of Head on gastric sensibility, though they indicate a somewhat wider spinal distribution of gastric afferent fibres than he supposed.

It is suggested, as a consequence of this study, that the movements and attitudes occurring in visceral diseases constitute a reflex picture of defense, namely, an endeavour, more or less effective, on the part of the organism to protect itself against harmful visceral irritation. The advantage of drawing up the legs, a symptom commonly met with in such conditions, is that, thereby, the irritated viscus is subjected to less pressure.

Demonstration of stimulation of the gastric glands by mechanical distention of the stomach with specimens and roentgenograms of a pouch of the entire stomach. A. C. Ivy, R. K. Lim, J. P. McCarty, J. I. Farrell, University of Chicago.

A demonstration of roentgenograms and specimens was made showing that a pouch of the entire stomach had been made and that no gastric mucosa was left at the site of the end-to-end duodeno-esophageal anastomosis. (We thank Dr. Carl Pfender of Washingoon, D. C., for making roentgenograms of the dog demonstrated in Washington.) A dog with a pouch of the entire stomach and a duodeno-esophageal anastomosis was demonstrated. The dog was in an excellent state of nutrition and when the stomach was distended with a toy balloon (200 cc.) the gastric glands were stimulated.

Mobilization of salt and water before sweating, as determined by the specific

gravity: solids ratio of blood and serum.[1] H. G. BARBOUR, W. F. HAMILTON, M. H. DAWSON and I. NEUWIRTH, University of Louisville.

The phase of blood dilution in response to warm environments which occurs in man before and early in the sweating process has been found best exhibited when the "wet-kata cooling-power" of the environment is kept in the neighborhood of eight or nine millicalories.

Healthy young men clad only in athletic underwear have been exposed to such conditions five or six hours after a light breakfast. Dilution of both whole blood and serum is best exhibited during the first hour in the warm room. The hemoglobin is most affected; it may decrease by ten per cent or more. The solids of both blood and serum are constantly diminished but by only two or three per cent of their total amount. They increase again when sweating starts. The diminution in specific gravity[2] of both blood and serum lags behind the solid content and may even show an initial increase.

The tendency of the $\frac{specific\ gravity}{solids}$ ratio to increase just before the sweating process, was taken to indicate the mobilization of salt as well as water. As it is relatively more pronounced in serum than in whole blood, the first source of sweat appears to be the blood cells.

Salt mobilization has in five cases been demonstrated by following the ash content of the serum. In every case the increase began within half an hour; the average maximum ash increase amounted to 0.06 (varying from 0.03 to 0.08) per cent. Serum nitrogen was determined in four cases and the protein loss thus established in the dilution phase was found greater than could be accounted by the total solids. A mobilization of lipoids is therefore indicated. The lipoids were estimated by assuming dextrose and n.p.n. constituents constant in the following equation: total solids = protein + ash + lipoids + dextrose + n. p. n. substances.

On this basis the serum lipoid increase averaged fifty per cent in four cases. It is probable that fatty substances are thus mobilized for the sebaceous secretion which accompanies sweating. Further evidence of lipoid mobilization was seen in a specific gravity decrease observed as sweating began, which was too great to be accounted for in any other way.

In regulation against heating the increased peripheral flow may result in nearly complete oxygen saturation of the venous blood (Meakins). This favors a shift of water and salts from cells to serum (Van Slyke, Wu and Maclean). Our results accord with the hypothesis that this constitutes the first step in the mobilization of sweat.

Experiments on renal blood flow with a description of two new methods for its determination. I. A. E. LIVINGSTON. II. G. W. WAGONER and A. E. LIVINGSTON, University of Pennsylvania.

This work was begun for the purpose of confirming and extending the observations of Richards and Plant in which intravenous injection of minute doses of renal vaso-constrictor substances was found to cause coin-

[1] Preliminary report of investigations receiving support from the Ella Sachs Plotz Foundation.

[2] Determined by the falling drop method. (Barbour and Hamilton: This Journal, 1924, lxix, 654.)

cident swelling of the kidney, decrease in renal blood flow, and diuresis—a result which was interpreted to mean constriction of vasa efferentia.

I. The first method is a modification of the one described by Brodie (1907). Any organ, which can be enclosed in a plethysmograph with its outflow arranged in such a manner as to permit temporary clamping, lends itself to this method. The plethysmograph is attached to a sensitive Brodie bellows by which the sudden increase in volume is recorded when the vein is automatically closed by a magnetic clamp for a definite length of time. The clamping is intermittent; alternate periods of one-half and one second have usually been chosen. The volume increase is read directly off the record by means of a scale calibration of the bellows in fractions of a cubic centimeter, thus giving a direct reading of blood flow in terms of cubic centimeters per second.

II. The second method utilizes the principle of the Venturi meter as used in hydraulics.

The first device which we used consisted of a small bent glass tube, drawn out at one point so that its calibre is narrow and having one end shaped for insertion into the stump of the superior mesenteric artery, the other into the central stump of the aorta just peripheral to the origin of the left renal artery. Attached to this tube are two side tubes, one at the point of greatest constriction, the other at an unconstricted point nearer to the mesenteric artery. These two tubes are connected at the top so that they form a differential manometer. The difference in height of fluid columns in these two tubes varies with the volume flow through the glass tube. After the tube has been inserted in the manner just suggested, the aorta is tied between the origin of the superior mesenteric artery and that of the left renal artery. All of the arterial blood which reaches the left kidney must pass through this meter, and volume of blood flow is determined by frequent readings of differential pressure as shown in the manometer. The instrument is calibrated by allowing known volumes of an acacia solution of the same viscosity as blood to flow through it at different rates. A curve of differential pressures corresponding to different rates of flow is constructed, and used for reading the volume flow of blood in the actual experiment.

A further development consists of two vertical tubes, containing 10 per cent citrate, inserted into the stump of the coeliac and superior mesenteric artèries and connected at the top. A ligature around the aorta just tight enough to form a throat at the point of origin of the latter transforms the aorta itself into an instrument similar to the glass one just described. When it is desired to measure flow through the kidney it is necessary to tie all vessels peripheral to the meter except the renal artery. The aorta thus used as a meter is just as sensitive as the glass one for relative flows, but presents greater difficulties in calibration for absolute flows. The aorta with surrounding tissue, tubes, and ligature is excised at the end of the experiment and calibrated in the same manner as the glass tube. In this case the injection of an anticoagulant is not required as it is when the glass tube is inserted into the circulation.

The results of Richards and Plant have been confirmed by these methods, and observations are now in progress on the question of anuria following anemia of the kidney as recently investigated by Marshall, Carlson and others. The results thus far obtained indicate that anemia of the kidney

of fifteen minutes' duration is not followed by prolonged anuria if the anemia is produced without mechanical disturbance of the nervous mechanism in the region of the renal artery.

Experimental data relating to the chemical regulation of respiration. ROBERT GESELL, D. W. BRONK (by invitation), D. A. MCGINTY (by invitation), A. B. HERTZMAN (by invitation), F. W. BALD (by invitation), F. H. LASHMET (by invitation), R. P. MONTGOMERY (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

F. H. LASHMET. The lack of correspondence between the hydrogen ion concentration inside and outside the red blood cell was studied by the oxygen combining power of hemoglobin. Cells suspended in two artificial plasmas of the same osmotic pressure and the same pH but containing different amounts of H_2CO_3 and $NaHCO_3$ combined with less oxygen in the plasma containing the larger amount of H_2CO and $NaHCO_3$. Omitting H_2CO_3 from the plasmas, the cells suspended in the plasma containing the larger amount of $NaHCO_3$ combined with the lesser amount of oxygen. The results appear explainable by the impermeability of the red cell membrane to the metallic cations.

R. P. MONTGOMERY. A similar study was made on the sartorius muscle of the frog using the development of fatigue to tetanic stimulation in alternate solutions as an index to the acidity of the inside of the cell. Fatigue developed more rapidly when the muscle contracted in the solutions containing the greater amount of $NaHCO_3$ whether H_2CO_3 was present or not. It is tentatively suggested that H_2CO_3 and temporarily $NaHCO_3$ exert an acid effect from without on intracellular acidity of the contracting muscle. The effects of the solutions on the height of the initial contractions indicate that the muscle cell is permeable to the sodium ion.

F. W. BALD. Moderate hemorrhage in the decerebrate dog was followed by increased pH of the arterial blood which was usually accompanied by increased pulmonary ventilation. The pH of the arterial blood, however, may increase in the absence of increased pulmonary ventilation. Injection of gum-saline solution was followed by decreased pulmonary ventilation and decreased pH of the arterial blood. Severe hemorrhage was associated with decreased pH regardless of the extent of pulmonary ventilation.

D. A. MCGINTY. The hyperpnea of the dog subjected to high oxygen pressure was associated with a lowered carbon dioxide content and a lowered pH of the arterial blood.

ROBERT GESELL and A. B. HERTZMAN. A continuous method for recording changes in the pH of the circulating arterial and venous blood with the use of the manganese dioxide electrode was developed. The continuity of the method, the facility of recording rapid changes in pH, the possibility of securing a large amount of data from single animals and obtaining synchronous records of pH changes in the arterial and venous blood along with records of pulmonary ventilation, oxygen consumption, blood pressure, etc., make the method extremely valuable.

Experiments performed on the effects of the administration of carbon dioxide, the intravenous injection of $NaHCO_3$ and Na_2CO_3, mechanical obstruction to respiration, the administration of rarefied air, the injection of NaCN, and on hemorrhage with subsequent injection of gum-saline

solution all showed characteristic changes in pH of arterial and venous blood.

The data support our view that there is no constant relation between pulmonary ventilation and the composition of the blood; that the acid metabolism of the respiratory center and the transport of acid are important factors. This is indicated by the striking effects produced by changes in the volume flow of blood on pulmonary ventilation regardless of the absence of change or direction of change of the composition of the blood.

Some effects of the vagus nerves upon intra-auricular block. WALTER E. GARREY, Tulane University, New Orleans.

In confirmation of earlier reports it is found that in nine species of turtles the usual effect of vagus stimulation is to enhance the degree of block produced by a clamp which compresses auricular tissue. Exceptions to this rule have been noted, not only when there is slowing of rate as previously noted but also without change of rate, as reported by Gaskell and by Lewis and Drury. The newer results show the decreased block due to vagus stimulation to be a transitory effect obtained under the following conditions: 1, as an after effect of stimulation of short duration and preceded by a definite interval of enhanced block; 2, less commonly after the stimulation has ceased but without evidence of previous increase of block; 3, infrequently the block is decreased during the vagus stimulation, sometimes preceded by increase of block, sometimes not; 4, occasionally a mild grade of block is immediately relieved when the neck vagus is stimulated; 5, rarely the decrease in the degree of block, under the above conditions persists as a permanent feature. The results stress the dual influence which the vagi exercise upon heart block. Both influences may appear in the same heart depending upon the conditions of stimulation.

The phenomena presented in the experiments suggest that there are other factors to be considered besides the shortening of the refractory period stressed by Lewis and Drury. Among these are mechanical adjustments between tissue and clamp, the rest factor, partial function of musculature, compression of nerve fibers, changes in the electrical state at the compressed region and the probable presence of cardio-augmentor fibers in the cervical vagus trunk.

A comparison of the basal metabolism of normal women with present prediction standards. GRACE MACLEOD and MARY SWARTZ ROSE, Department of Nutrition, Teachers College, Columbia University.

Determinations of the oxygen consumption in the post-absorptive state, the subject lying still, awake, after 25 to 35 minutes' rest, have been made on 92 normal women between the ages of 20 and 50. The Benedict Portable Respiration Apparatus was used. In all, 136 determinations have been made, with records of pulse, respiration rate and vital capacity. Comparisons have been made between the actual energy expenditure as calculated from the oxygen consumption and that predicted by the Aub-DuBois standards; by the Harris-Benedict formula; and by the Dreyer formula. The results are shown in the following table.

TABLE 1

Actual results with their deviations from predicted values

AGE	NUMBER OF PERSONS	CALORIES PER SQUARE METER PER HOUR	CALORIES PER KILO PER HOUR	CALORIES PER 24 HOURS	DEVIATIONS FROM PREDICTED VALUES					
					Aub-DuBois		Harris-Benedict		Dreyer	
					Without regard to sign	With regard to sign	Without regard to sign	With regard to sign	Without regard to sign	With regard to sign
20–29	42	33.8 ± 0.25	23.0	1307	10.2	−8.5	8.2	−5.4	8.1	−4.8
30–39	31	34.0 ± 0.29	23.1	1307	8.0	−6.9	6.7	−2.4	7.2	−1.0
40–49	13	31.3 ± 0.57	20.2	1244	14.1	−12.9	10.2	−6.8	10.1	−4.8

Table 2 shows the distribution of the cases with respect to their agreement with the predicted values.

TABLE 2

Distribution of cases with respect to agreement with prediction values

DEVIATION	AUB-DUBOIS		HARRIS-BENEDICT		DREYER	
	Number of cases	Per cent of total cases	Number of cases	Per cent of total cases	Number of cases	Per cent of total cases
per cent						
0–3	28	20.3	46	33.3	36	26.1
0–5	47	34.1	57	41.3	59	42.7
0–7	62	44.9	72	52.2	79	57.2
0–10	79	57.3	96	69.5	93	67.4
0–15	110	79.7	121	87.7	121	87.7

Pulse and respiration rates showed wide variation:

AGE	PULSE	RESPIRATION
years		
20–29	54–88	8–20
30–39	58–86	7–22
40–49	56–82	8–18

No correlation was observed between pulse rate and basal metabolism.

The deviations from Dreyer's standards for vital capacity are given in table 3.

TABLE 3

Vital capacity of normal women—Deviations from Dreyer's standards

AGE	DEVIATION FROM STANDARD VITAL CAPACITY	
	Without regard to sign	With regard to sign
years	*per cent*	*per cent*
20–29	10.6	−2.7
30–39	13.6	−4.5
40–49	16.4	−11.5

So-called "reversed" hemolysis, with further observations on the mechanism of hemolysis. META L. SCHROEDER (by invitation) and G. N. STEWART, Western Reserve University Medical School.

An apparent re-accumulation of blood pigment in the stromata of erythrocytes laked in various ways has been described by a number of observers, some of whom have held that the hemoglobin actually reënters the ghosts or is accumulated on them under the influence of salt solutions of various concentrations and in other ways. More than 20 years ago it was shown by one of us[1] that mammalian corpuscles and still better the large erythrocytes of Necturus[2] when fixed by formaldehyde to such a degree that actual exit of blood pigment does not occur on heating in a dilute solution of ammonia, become pale and *appear* to be laked on heating in the ammoniacal water, and to re-accumulate the pigment when subsequently acted upon by hypertonic solutions. It can be shown very easily that these appearances are entirely due to the redistribution of the pigment within the corpuscles, the concentration diminishing when they swell and increasing when they shrink. We have repeated these observations on the erythrocytes of Cryptobranchus with the same results. Crystallization of hemoglobin, either outside or inside the erythrocytes, was not observed under the influence of laking reagents (on the unfixed erythrocytes of Cryptobranchus), although so striking in Necturus erythrocytes.

In experiments on rabbit's blood (either the defibrinated blood or suspensions of washed erythrocytes in salt solution, or both) we have determined: *a*, the electrical conductivity of the blood or suspension, of the serum or suspending liquid, and of the sediment before and after laking with sodium oleate, saponin or heat; *b*, the hemoglobin concentration in the same fractions; *c*, the specific gravity of the same fractions.

From the conductivities we have calculated the minimum proportion of the total volume of the laked suspensions (or blood) which must have been occupied by the ghosts (32 per cent, 26 per cent, 17 per cent, 18 per cent and 28 per cent) in 5 experiments in which the corresponding numbers for the volume of the erythrocytes in the unlaked suspension was 32 per cent, 30 per cent, 31 per cent, 30 per cent and 54 per cent respectively. An attempt was made to adjust the intensity of the laking process so as to give approximately complete laking and no more, and the microscopic "reversion" reaction was well obtained. The calculated volumes were checked by hematocrit readings, although for obvious reasons the degree of accuracy obtainable with the hematocrit for ghosts is still less than for erythrocytes. The hemoglobin concentrations were practically the same for the laked suspensions, the suspending liquids separated from them and free from ghosts and the sediment of ghosts.

The specific gravities were very nearly the same for the suspending liquid of the laked material free from ghosts, the laked suspension and the sediment of ghosts. But, as follows from the fact that sedimentation was obtained by the centrifuge, they increased in the order named.

From the substantial proportion of the total volume occupied by the ghosts, according to *a* (with mild laking agents such as sodium oleate sometimes not less than that occupied by the unlaked erythrocytes) and the findings under *b* and *c*, it follows that the volume of the ghosts in the

[1] Journ. Med. Research, 1902, viii, 276.
[2] This Journal, 1902, viii, 121.

laked suspension must be made up mainly of approximately the same hemoglobin solution as that in which they are suspended. After complete laking, therefore, they contain a large amount of the hemoglobin, and the apparent "reversal" of the hemolysis when they are acted upon by solutions of various substances is due to an alteration in the condition (concentration) of the hemoglobin already within them and not to the taking up of hemoglobin from the outside.

Eyeball reflex movement associated with voluntary and reflex winking. W. R. MILES, Stanford University.

The recording of eye movements by means of photographing the corneal reflection (high-light) from the crater of a carbon arc lamp, is a technique that has been in use for several years. Thus far it has been employed almost exclusively in studying horizontal eye movements as performed in reading English or texts similarly arranged. Recently at Stanford University records were made in connection with the reading of vertically printed Chinese, and in these photographs a peculiar movement of the eyeball was found to occur at moments of winking. Hints of this reflex movement had been noted by the writer on records taken in 1914.

The coördination of winking was studied directly by attaching small, very light-weight convex mirrors to the upper and lower eyelids respectively so that bright high-lights could be obtained for photographing from the lids as well as from the cornea of the eyeball. The film was moved in the horizontal. The magnification was 5 times and the beam of light was interrupted by a timing motor so as to record in units of 0.01 second.

It was found that synchronously with the lowering of the upper lid there was a very rapid elevation of the eyeball with a much slower reverse movement when the lid was lifted. This movement of the eyeball appears in both reflex and voluntary winking and seems not to depend on a condition of sleepiness. In cases where the lid reflex is small, practically the complete curve for the cornea movement is recorded. The size of the movement is in the order of 10 to 15 degrees on the arc of vision. Records taken on still plates show that the shift is upward and inward; however, the lids in their movements at the time of winking have much more prominent lateral displacements toward the inner angle. The opposed telescoping movement of the eyeball and upper lid obviously results in a more prompt protective covering for the cornea. To what extent this shifting of the line of regard modifies the efficiency of vision has not been determined. Under certain lighting conditions one can easily notice the shift in the stimulus, a tailing off downward, at the instant of winking. This eyeball movement has probably been observed in cases of Bell's palsy and seems related to the well-known "Bell phenomenon" or upward roll of the eyes during sleep.